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[This is a full translation of selected articles in Meditsinskaya Radiologiya (Medical Radiology) Vol IV, No 10, 1959, pages 17-46, 59-95.]

TABLE OF CONTENTS

ANNALS

The Effect of Local Irradiation on the Concentration of Acid-Insoluble Phosphorus Fractions in the Bone Marrow of Rabbits

V. S. Turovskiy

From the Department of Biochemistry (Head -- Corresponding Member of the Academy of Medical Sciences USSR Professor V. S. Il'yin) of the Institute of Experimental Medicine of the Academy of Medical Sciences USSR

The effect of total-body radiation on the concentration of nucleic acids and phospholipids in the bone marrow has been studied by Lutwak-Mann, who showed that total-body irradiation of rats (500 r) leads to the marked reduction in the concentration of desoxyribonucleic acid and a reduction in the concentration of ribonucleic acid. Complete recovery of the concentration of nucleic acids in the bone marrow of irradiated rats occurred after 48 days. According to the data of Martin and others, six to eight hours after a total-body irradiation of rats in a dose of 800 r the uptake of P^{32} in the nucleic acids of the bone marrow is reduced by 43 percent.

Thomson and others have shown that local irradiation of the hind extremity of a rabbit with γ-rays of Co^{60} in doses of 310 and 620 r leads to a marked reduction in the nucleic acid concentration in the irradiated bone marrow after 48 hours, whereas the concentration of phospholipids is practically unchanged in it.

R. Ye. Libinzon studied the concentration of nucleic acids and phospholipids and the uptake of P^{32} in these fractions of the bone marrow of rabbits and found that the concentration of nucleic acid phosphorus and the uptake of P^{32} are markedly reduced after a total-body irradiation of rabbits with a dose of 1,000 r. The concentration of phosphorus in the phospholipids did not change very much after the irradiation. Ye. A. Dikovenko found that both after total-body irradiation of rabbits in a dose of 300 and 2,000 r and after local irradiation of the head (600 and 4,500 r) and of the abdomen (2,000 r), a reduction in the concentration of nucleic acids is observed and of the uptake of P^{32} in them.

In the present work, a study was made of the concentration of acid-insoluble phosphorus fractions: nucleic acids, phosphoproteins and phospholipids and the rate of uptake of P^{32} in them in the bone marrow of an irradiated rabbit extremity ("direct" effect of radiation) and in the bone marrow of a non-irradiated, lead-protected extremity ("reflected" effect). The same indices in the bone marrows of non-irradiated rabbits served as the controls.

For the purpose of irradiating the animals, an RUM-3 apparatus was used. The voltage was 180 kv, the anode current was 15 ma, the turret with the radiation field was 10 x 15 centimeters, filter of 0.5 mm Cu and one mm of Al, distance from the anode was 30 centimeters, dose rate in air was 67.3 r/min. The entire body of the rabbit, with the exception of the left hind extremity, was covered with lead three millimeters thick. The dose rate was 500 r. Phosphate ($Na_2HP^{32}O_4$) was injected intravenously according to the calculation of 50,000,000 impulses per minute (0.4 mC/kg).

Using our dosages the isotope administered could in itself exert an influence on the metabolism of bone marrow phosphorus compounds. However, we could not find any changes in the morphological picture of the bone marrow or peripheral blood in control experiments (without irradiation). Twenty-four hours after the injection of P^{32} the rabbit was beheaded. Treatment of the material was made according to the Schmidt and Tannhauser method in the Schneider modification. The magnitude of the turnover, that is, the number of impulses for the given function per 100 grams of bone marrow tissue (product of the specific activity and the concentration in milligrams-percent) was computed.

In control experiments, a study was made of the concentration of acid-insoluble phosphorus fractions, their specific activity -- the number of impulses per milligram of phosphorus of the fraction being studied and the magnitude of the turnover in the bone marrow of normal rabbits 24 hours after the injection of P^{32} into them (this period was established as a result of special experiments).

The results of the experiments showed that local irradiation of a single extremity of the rabbit led to a change in the concentration of phospholipids in the bone marrow of both extremities. The phospholipid concentration in the irradiated extremity decreased markedly 24 hours after irradiation (by 34.8 percent), and during the next three days remained at the same level. Beginning with the fourth through the tenth day after irradiation the concentration of phospholipids returned to the control level (99.4 percent); beginning with the 10th through the 32nd day it continued to increase, and on the 32nd day it exceeded the control by 82.3 percent.

Different dynamics were observed under the influence of the reflected effect of irradiation. The concentration of phospholipids in the bone marrow of the non-irradiated extremity 24 hours after irradiation was no different from the control, but after 48 hours it increased notably (by 27 percent), and on the 32nd day after irradiation was 70.8

percent higher than the control. The rate of P^{32} uptake in the phospholipids of the bone marrow of irradiated and non-irradiated extremities changed in approximately the same way -- it was somewhat reduced during the entire time of the observation.

The difference in the nature of metabolism of the phospholipids in the bone marrow of the radiated and non-irradiated extremities was shown by comparison of the values of the turnover of this fraction. The magnitude of the turnover rate of phospholipids in bone marrow decreased both after the direct and reflected effects of irradiation, but this decrease was considerably more marked in the bone marrow exposed to the direct effect of X-rays. The reflected effect produced a change in the turnover rate in the same direction but less marked and of shorter duration. The rate of turnover of phospholipids began to return to normal 24-48 hours after irradiation in the bone marrow exposed to the reflected effect, reached the control level, and on the 10th day after irradiation considerably exceeded it (by 65.4 percent). The return to normal of the rate of phospholipid turnover of the directly irradiated bone marrow began only four to ten day after irradiation, reaching the control level (104.6 percent) on the tenth day.

In the Table results are presented of the direct and reflected effects of irradiation (500 r dose) on the concentration, specific activity, rate of phosphoprotein turnover in the bone marrow.

The phospholipid concentration in the bone marrow 24 hours after the direct effect of X-rays decrease considerably (by 44.2 percent compared to control). In the subsequent period (48 and 96 hours after irradiation) the phosphoprotein concentration increased somewhat; however, it was far from reaching the control level, remaining 30.2 percent and 25.5 percent below it. After four to ten days the concentration of phosphoproteins increased markedly, and on the tenth day it notably exceeded the control (by 20.9 percent). The changes in phosphoprotein concentration after the reflected effect of irradiation were of the same nature but were much less pronounced. The changes in specific activity of the phosphoproteins in the irradiated and non-irradiated bone marrow of experimental animals were similar.

The nature of the changes of the concentration in the nucleic acids in the bone marrow under the influence of irradiation was similar to the phospholipid changes. Direct irradiation produced a marked reduction in the concentration of nucleic acids in the bone marrow. The reduction was equal to 41 percent 24 hours after the irradiation and remained at a low level up to ten days. Only between the

The Direct and Reflected Effect of Irradiation in a Dose of 500 r on the Bone Marrow Phosphoprotein (Average Figures in Percentages of Control)

Day after irradiation	No of experiments	Direct effect of irradiation			Reflected effect of irradiation		
		Concentration	Specific activity	Rate of turnover	Concentration	Specific activity	Rate of turnover
1st	8	55,8	44,7	24,4	74,5	57,3	46,1
2nd	18	69,8	36,4	22,6	93,0	64,2	56,4
4th	8	74,5	43,9	28,0	120,4	70,4	76,8
10th	8	120,9	56,0	66,1	167,4	66,7	116,5
32nd	8	239,5	53,0	135,7	297,6	54,8	154,3

10th and 32nd day did a rapid recovery in the nucleic acid concentration begin, and by the 32nd day it considerably exceeded the control. R. Ye. Libinzon found that a marked reduction in the nucleic acid concentration and rate of uptake of P^{32} in them after total-body irradiation of rabbits in a dose of 100 r occurs on the third to fourth day after irradiation. This observation coincides with ours only with respect to local irradiation of the bone marrow. After the reflected effect of irradiation an increased concentration of nucleic acids in the bone marrow was observed by us throughout the entire period of study.

Changes in the specific activity of bone marrow nucleic acids were of an opposite nature after the direct and reflected effects of the irradiation. The direct effect of irradiation was accompanied by a notable reduction in the rate of uptake of P^{32} in the nucleic acids (by 28 percent after 48 hours), after which the specific activity began to increase, and during the period between the fourth and tenth day it returned to normal, and then exceeded the control. After the reflected effect of irradiation the specific activity of nucleic acids increased from the very beginning and continued to increase during the subsequent period of the observation.

Naturally, changes in the values of the rate of nucleic acid turnover, which depend both on their concentration and on the specific activity, were of an opposite nature

after the direct and reflected effects of irradiation: in the former case it was markedly reduced; in the latter, markedly increased.

According to its nature the direct effect of irradiation on the rate of turnover of all three fractions may be divided into two periods: 1) the period which lasted for the first two weeks after irradiation, during which a decrease occurred in the rate of acid-insoluble phosphorus fraction turnover; 2) the recovery period, which began between the fourth and tenth days after irradiation and led not only to complete restoration of the rate of the turnover of all three fractions but also to a considerable increase of it over the control level with respect to nucleic acids and phosphoproteins. It is not possible at the present time to give a satisfactory explanation to such a marked "reactive" increase in the concentration of phosphoproteins, phospholipids and nucleic acids in the bone marrow in the remote periods after irradiation. It may be noted only that during this period a considerable restoration to normal occurs in the cellular composition of the bone marrow (Heineke, Wünsche, P. D. Gorizontov).

Our experiments showed considerable changes in the intensity of turnover of the acid-insoluble phosphorus fractions in the bone marrow of the second, non-irradiated extremity (reflected effect). With respect to phospholipids and phosphoproteins these changes also can be divided into a period of reduction and a recovery, or reactive period. With the reflected effect of the irradiation the period of reduction in the intensity of phospholipids and phosphoprotein turnover was not so pronounced and brief but the recovery period began earlier and the increase in the intensity of turnover of these fractions reached a higher level. The intensity of nucleic acid turnover in the non-irradiated extremity began immediately to increase sharply, principally because of an increase in the specific activity of this fraction. Therefore, after the reflected effect of irradiation, the period of reduction with respect to nucleic acids was not seen.

Conclusions

1. Irradiation with a dose of 500 r of the hind extremity of rabbits produces changes in the concentration and rate of turnover of the acid-insoluble phosphorus fractions in the bone marrow of irradiated ("direct" effect) and non-irradiated ("reflected" effect) of the extremity.

2. Direct irradiation first leads to a marked and prolonged reduction in the concentration and intensity of

phospholipid, phosphoprotein and nucleic acid turnover, after which (usually between the fourth and tenth day) the recovery period begins, and by the end of this period (32nd day) their concentration and rate of turnover exceed those in the bone marrows of non-irradiated rabbits.

3. The reflected effect of irradiation is characterized by a considerable increase in the concentration of phospholipids and nucleic acids after a brief and slight reduction in the concentration of phosphoproteins. The intensity of phospholipid and phosphoprotein turnover, after the brief and slight reduction, increases, whereby this increase is expressed to a much greater extent than after the direct effect of irradiation.

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The Effect of the Adrenocorticotropic Hormone of the Hypophysis and Suprarenal Cortical Extract on Hematopoiesis in Irradiated Animals

M. F. Aleksandrova

The aim of our work was to study the effect of the adrenocorticotropic hormone (ACTH) of the hypophysis and of suprarenal cortical extract on hematopoiesis in irradiated animals. One hundred and eighty-six male white rats weighing 180-230 grams were used for the work.

The animals were divided into three series: rats of the first series were not irradiated; rats of the second series were irradiated on an RUM-3 apparatus; rats of the third series, on a 12-tube X-ray apparatus under standard technical conditions. The entire work was carried out with Soviet preparations: suprarenal cortical extract (cortin) and the long-acting hypophyseal adrenocorticotropic hormone (ACTH zinc phosphate), the method for obtaining which had been worked out in the All-Union Institute of Experimental Endocrinology (N. M. Rudenko). The preparations were injected intramuscularly for three weeks; ACTH, in a dose of five units twice a week; cortin, in a dose of 2.5 units twice a day.

In all the rats the peripheral blood was examined for two months (concentration of platelets, reticulocytes, erythrocytes, hemoglobin, leucocytes, and the differential white blood count) and an investigation was made of bone-marrow and spleen preparations.

The Effect of ACTH on the Hematopoiesis of Rats Following a Total-Body Single Irradiation With a Dose of 400 r

Very substantial differences were observed in the hematopoietic reaction of rats which had been injected with ACTH beginning with the first day after irradiation by comparison with the irradiated control animals. An extremely insignificant relative increase was noted in the concentration of reticular cells. Apparently, the capacity of them for being differentiated into blood cells was impaired to a lesser degree than in the control irradiated rats (Fig. 1a). ACTH exerted a normalizing effect also on the maturation processes of erythroblasts and bone-marrow granulocytes. In Fig. 2c it is seen that the maturation index of erythroblasts is unchanged on the fifth day during a period of maximum changes. The maturation curves of erythroblasts and granulocytes of animals (Figs. 2a and 2b) which were given

ACTH after irradiation are of the same nature as in the normal animals. A certain activation of the regeneration processes may also be noted. While mitoses of both the red and white blood were absent in irradiated rats on the tenth day, after the injection of ACTH they were found in quantities which somewhat exceeded the level in normal animals.

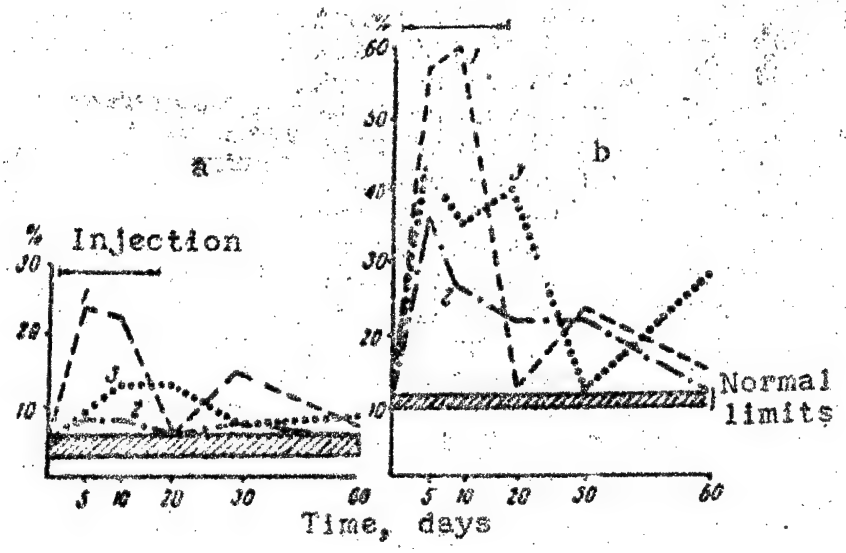

Fig. 1. Concentration of Reticular Cells in the Bone Marrow (a) and Spleen (b) in Rats.

1 -- 400 r; 2 -- 400 r plus ACTH; 3 -- 400 r plus cortin.

ACTH had a favorable effect on the spleen also. The increase in the content of reticular cells was not so great (Fig. 1b), and the degeneration was reduced (see Fig. 3). During the period of administration of ACTH, the concentration of lymphoblasts and large lymphocytes was within normal limits, whereas in the control rats it decreased (Fig. 3b). The spleen was richer in cellular elements than in the control animals. All this led to the fact that changes in the peripheral blood were less pronounced. The red blood count decreased by an average of 15 percent by the 15th day (in controls, by 40 percent) (Fig. 4), recovery was more active, and the red blood cells returned to their original level by the 25th-30th day (in the control, by the 45th day). The degree of reduction in the leucocyte concentration was the same as in the control animals, but recovery occurred in a shorter time. The increase in the concentration of leucocytes occurred chiefly because of the increase in neutrophils, but it is interesting to note that the recovery in the lymphocyte count also was more active.

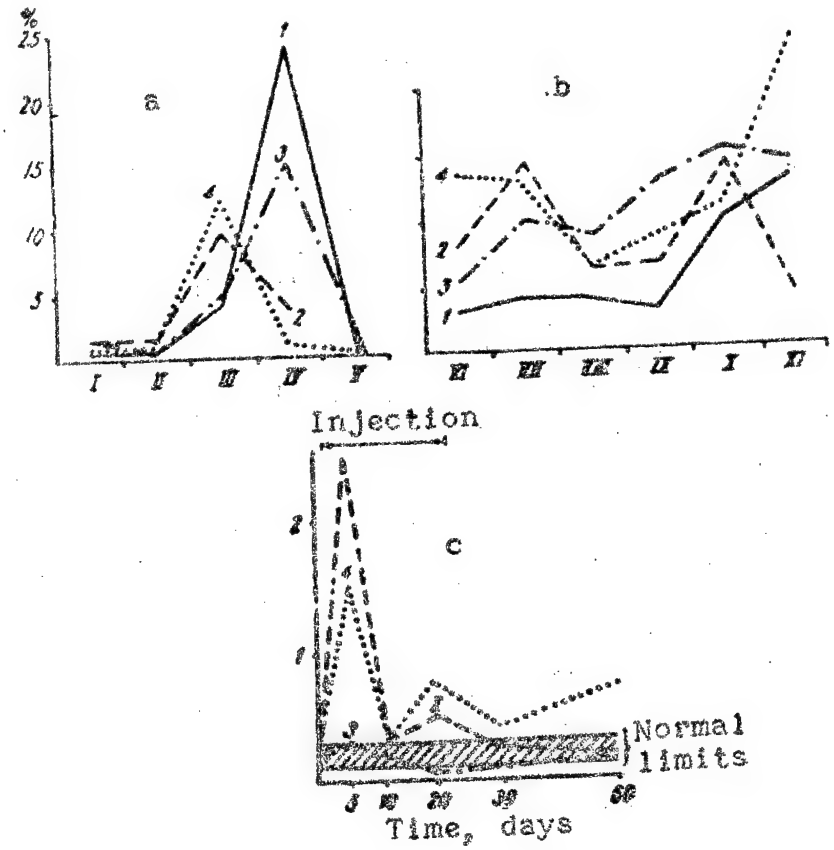

Fig. 2. Maturation Curves of Erythroblasts (a) and Granulocytes (b) of Rat Bone Marrow on the Fifth Day After Irradiation (in Percentages of the Total Number of Bone-Marrow Cells); Maturation Index of Erythroblasts on the Fifth Day after Irradiation (c).

1 -- normal; 2 -- 400 r; 3 -- 400 r plus ACTH; 4 -- 400 r plus cortin; I -- proerythroblasts [hematoblasts]; II -- basophilic macroblasts [megaloblasts]; III -- basophilic normoblasts; IV -- polychromatophilic normoblasts; V -- eosinophilic normoblasts; VI -- myeloblasts; VII -- promyelocytes; VIII -- myelocytes; IX -- juveniles; X -- stabs; XI -- segmented neutrophils.

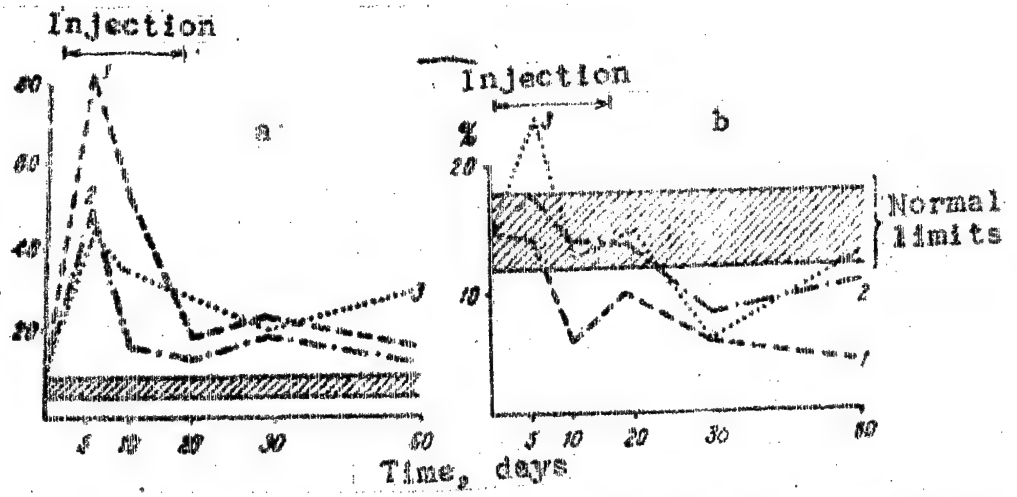

Fig. 3. Degeneration of Cells in the Spleen (a); Concentration of Lymphoblasts in the Rat Spleen (b).

1 -- 400 r; 2 -- 400 r plus ACTH; 3 -- 400 r plus cortin.

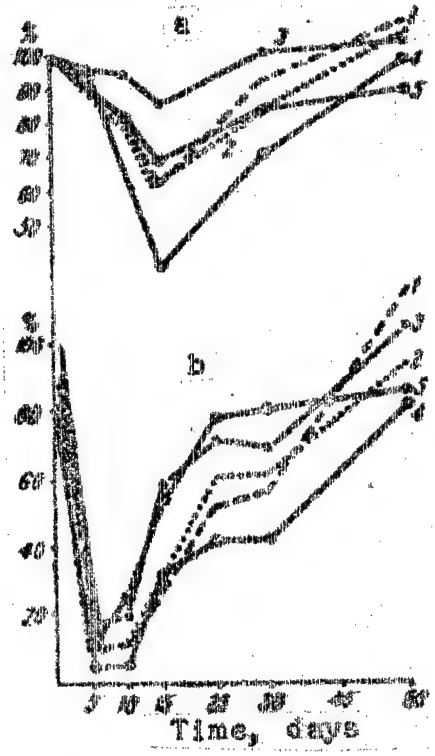

Fig. 4. Change in the Erythrocyte Concentration (a) and Leucocyte Concentration (b) in the Peripheral Blood of Experimental Rats.

1 -- 400 r; 2 -- 400 r plus cortin; 3 -- 400 r plus ACTH from the first day; 4 -- 400 r plus ACTH from the seventh day; 5 -- ACTH before irradiation.

Aside from this experimental variant, where ACTH was injected beginning with the first day after irradiation, we examined the blood of animals which had been given an injection of ACTH beginning on the seventh day after the irradiation (in a dose of five units twice a week for three weeks), or after a double injection of ACTH during the three days before irradiation. When the preparation was injected beginning with the seventh day we did not obtain any beneficial results; the erythrocytopenia and leucopenia were more severe (Fig. 4), and recovery was delayed. The injection of ACTH before irradiation somewhat lessened the degree of reduction in the erythrocyte count; however, the recovery of them also occurred in a delayed manner. The white blood count decreased just the same way as in the control animals, but recovery was more active.

The Effect of Cortin on the Hematopoiesis of Rats After a Total-Body Single Irradiation With a Dose of 400 r

Cortin contributed to a certain normalization in hematopoiesis of irradiated animals; however, it was less effective. In exerting a definite beneficial influence on the processes of transition of reticular bone-marrow cells and splenic cells into blood cells, cortin, in contrast to ACTH, did not act on the maturation processes of erythroblasts and granulocytes. In Fig. 2a and 2b it is seen that in animals which were given cortin injections after irradiation there was just as marked an inhibition of the maturation processes as in the control animals. In the spleen of the rats the concentration of lymphoblasts and large lymphocytes increased on the fifth day after the irradiation (by comparison with the normal), which was apparently brought about by a delay in the maturation processes of the lymphocytes. In accordance with this, the number of cellular elements in the spleen was reduced by comparison with animals which were given ACTH. Changes in the concentration of leucocytes and erythrocytes in the peripheral blood of animals of this group and of the controls were the same (Fig. 4).

The Effect of ACTH and Cortin on the Hematopoiesis of Rats After Fractional Irradiation With a Dose of 600 r (The Irradiation Was Carried Out for 50 Days).

In all (18) animals a reduction in the white blood count was noted by the end of the irradiation period (by 70-85 percent) and of the erythrocyte count (by 10-15 percent). After stopping the irradiation for three weeks, the

rats were given ACTH injections or cortin injections according to the system indicated above. No essential difference was noted in the recovery of the peripheral blood composition of control and experimental animals.

These investigations showed that the Soviet preparations of hypophyseal ACTH and suprarenal cortical extract (cortin) possess a high degree of activity and exert an influence on the hematopoiesis in both intact and irradiated rats. In the analysis of the data obtained we directed attention to the fact that in the reaction of the bone-marrow red blood series to the injection of these hormones there was a certain similarity with the reaction to irradiation. Thus, for example, the acceleration of erythroblast maturation, which was accompanied by an increase in the erythrocyte concentration in the peripheral blood was common to both; after irradiation, it is usually observed early, during the first few hours or in the first day (A. P. Yegorov and V. V. Bochkarev; M. F. Aleksandrova; M. S. Lapteva-Popova and others). In all animals there was also a relative reduction in the number of cells of the red blood series noted; in irradiated animals, from 25 percent in the normal to 13-14 percent on the fifth day; in rats which were given cortin injections, to 7-17 percent on the fifth day; in rats which were given ACTH, to 16-18 percent on the 10th day.

Both after the injection of these hormones and after irradiation, a disturbance occurred in the differentiation of reticular cells of the spleen into blood cells, an increase in the lysis of lymphocytes and a reduction in the number of cellular elements in the spleen.

It is known that the background against which this effect occurs exerts a considerable influence on the nature of the effect of the hormones (S. M. Leytes). As a result of the deep-seated changes produced in the body by irradiation the nature of the effect of hormones was considerably changed in our experiments compared with the effect on intact animals. Thus, while in intact rats after the injection of ACTH and cortin we observed an increase in lymphocytolysis in the spleen, relative increase in the reticular cell concentration and reduction in the total quantity of splenic elements, in the irradiated animals after the injection of the preparation lymphocytolysis decreased, and a certain normalization of the differentiation process of reticular bone-marrow cells and of splenic reticular cells into blood cells was noted. The number of cellular elements in the spleen of experimental rats was greater than in the control rats. At the same time, ACTH exerted a normalizing effect also on the maturation processes of blood cells and

stimulated the regeneration processes, which apparently also accounted for the fact that the injection of ACTH considerably decreased the degree of reduction in the peripheral blood erythrocytes and accelerated the recovery in the concentration of leucocytes and erythrocytes, whereas cortin did not exert any influence on the composition of the peripheral blood. Despite the fact that the injection of ACTH cortin somewhat decreased the degree of injury to hematopoiesis, we did not observe any reduction in the mortality rate in the experimental group compared with the control group.

Conclusions

1. The injection of ACTH once into irradiated rats reduces the degree of injury to hematopoiesis and contributes to a more active recovery of the concentration of erythrocytes and leucocytes in the peripheral blood. ACTH exerts a beneficial effect when it is injected beginning with the first day after irradiation.

2. The influence of suprarenal cortical extract on hematopoiesis is insignificant in rats irradiated once.

3. Injections of ACTH and cortin do not exert any influence on hematopoiesis in rats subjected to fractional irradiation with a dose of 600 r.

4. The effect of these preparations on hematopoiesis in intact and irradiated animals is different.

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Investigation of the Properties of Irradiated Erythrocytes by the Striction Method

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Quite a few facts have been described in the literature attesting to the formation of toxic products in isolated cells as well as in the tissues and organs of animals and plants subjected to the effect of ionizing radiation. At the same time, various authors mention features of similarity during the course of radiation sickness, on the one hand, and in the pathogenesis of certain toxic states, on the other.

Among such features common to both syndromes are the following; the presence of an incubation (latent) period, relationship of the course of the disease to the temperature, and, finally, the vigorous development of a pathological process in its terminal period (B. N. Tarusov). The appearance of toxic substances in the tissues and tissue fluids of irradiated organisms can, however, have pathogenetic significance only in the event the accumulation of these substances occurs continuously throughout the entire period of the development of the disease (V. N. Benevolenskiy).

This requirement at present is satisfied only by the so-called "hemolytic factor" which was first discovered by A. S. Mochalina in the livers of irradiated rats, and then studied in detail by Yu. B. Kudryashov and identified by him as an unsaturated fatty acid. Nevertheless, the problem of the appearance and change in the activity of toxic products formed after radiation injury may be important also in those cases in which these processes occur with extinction.

Actually, a break in some of the chains of radiation-chemical reactions is able to lead to the formation of substances which are the starting points for other processes which have already developed according to the stage law, that is, according to the law characteristic of chain reactions with side-chains.

The task of the present investigation was a clarification of the possibility of producing toxic products in a suspension of irradiated erythrocytes. With this aim in view, we made use of the striction method worked out by B. N. Tarusov for the purpose of detecting bacterial toxins in tissues and tissue fluids.

The erythrocytes of rat blood, stabilized by dry sodium citrate, were separated from plasma by centrifugation and triply eluted with physiological sodium chloride solu-

tion, each time for 10 minutes at 1500 revolutions per minute. From the eluted erythrocytes a four-percent suspension was prepared in physiological solution, which then was divided into two equal portions. One of them remained as a control; the other was exposed to the effect of γ-rays on a GUT-Co-400 apparatus [telegamma apparatus]. Doses in various series of experiments amounted to 42, 63 and 84 kr using the same dose rate, which was equal to 700 r/min.

The detector for showing the toxic substances in the irradiated erythrocytes was a muscle brei which had been prepared from the gastrocnemius muscle of the white rat. A 200-milligram sample was used for each experiment; it was put into a dilatometer which had first been filled with a four-percent erythrocyte suspension. The dilatometer was immersed in a water incubator at a temperature of $30\pm0.01^{\circ}$. The readings were made by means of a horizontal MIR-1 microscope every five minutes for 75 minutes.

As is seen from Fig. 1, putting the muscle brei into irradiated substrates leads to a definite inhibition of striction by comparison with the control here (as subsequent experiments showed) the degree of inhibition of striction did not depend on the magnitude of the dose used and remained practically constant within limits of the range of doses used. At the same time, no "sudden jumps," which indicate the formation of toxic products according to B. N. Tarusov's data (Fig. 1), were observed in any of the experiments performed.

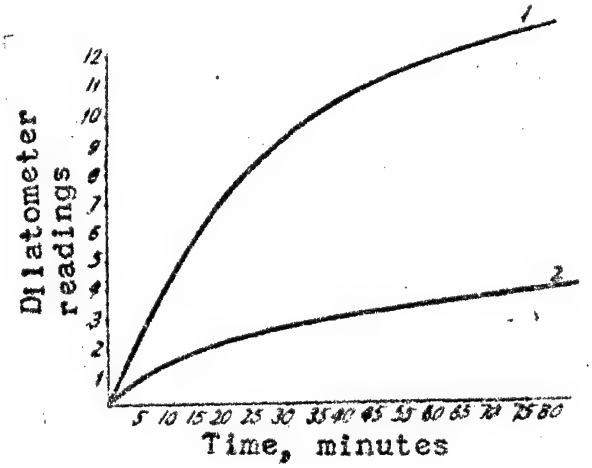

Fig. 1. Inhibition of Striction When Tissue Is Placed in Irradiated Suspension of Erythrocytes.

1 -- control; 2 -- experiment.

A completely different picture was observed when the

muscle brei was put into a suspension of irradiated erythrocytes after a one-hour incubation at 37°. In this case, as follows from Fig. 2, striking "jumps" occur on the curve characterizing the tissue striction in the irradiated substrate. The presence of these "jumps" can serve as proof of the occurrence of toxic products. With a less prolonged incubation (for example, 20 minutes), "the jumps" are not found. Subsequent experiments made it possible to establish the fact that "jumps" in experimental curves continue to be maintained even 9, 24 and 48 hours after the irradiation under conditions where the material is kept in a refrigerator at 3-5°. Thereby, the quantity and amplitude of the "jumps" increase with the increase in the time interval which has elapsed from the time of irradiation (Fig. 3).

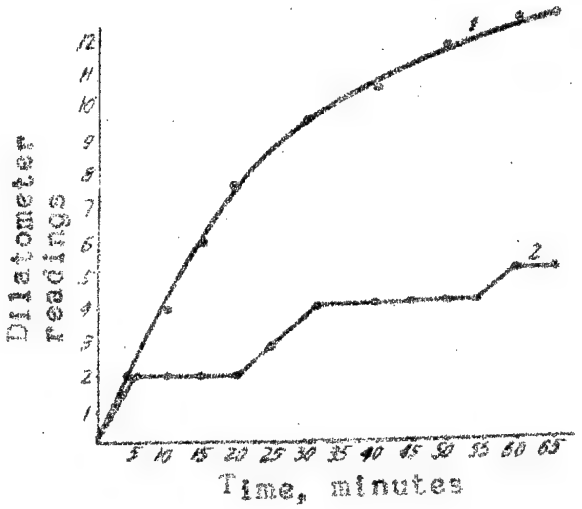

Fig. 2. The Occurrence of "Jumps" on an Experimental Curve After Irradiation and a One-Hour Incubation at 37°.

1 -- control; 2 -- experiment.

At the same time, another phenomenon could be noted -- a gradual "elimination" of striction inhibition, that is, an equalization of the ordinates of the control and experimental curves. Seventy-two hours after irradiation, the control and experimental curves could be superimposed on each other. By this time the "jumps" disappear, that is, the toxin-formation apparently stops.

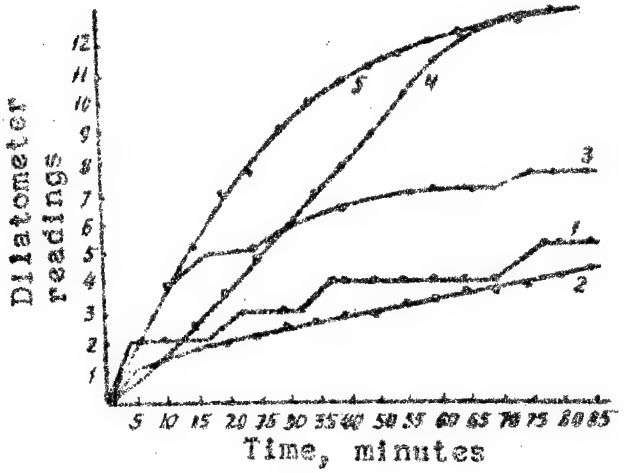

Fig. 3. Change in Striction Curves When Irradiated Erythrocytes Are Preserved in the Cold (3-5°).

1 -- one hour after irradiation; 2 -- nine hours after; 3 -- 24 hours after irradiation; 4 -- 48 hours after; 5 -- 72 hours after.

Naturally, the question rises: are the physicochemical processes described above specific for radiation and injury of erythrocytes or do they occur also in other forms of hemolysis? With the aim of clarifying this problem similar experiments were performed in which the erythrocytes were subjected to a preliminary distilled water or saponin destruction. In the former case, a four-percent erythrocyte suspension in distilled water was prepared. After 30 minutes, that is, on the completion of hemolysis, a sample of dry sodium chloride was added to the suspension so that the concentration of supernatant fluid corresponded to the isotonic. The preliminary one-hour incubation of such a suspension did not lead to a subsequent occurrence of "jumps" in the experimental curve. Negative data were obtained also after the reaction of the muscle brei with a four-percent erythrocyte suspension in 0.01 percent saponin solution prepared in physiological solution (Fig. 4). Therefore, the toxicometric test proved to be positive only after irradiation hemolysis and negative after other forms of it.

The second problem stemming directly from the first consisted in an elucidation of the location of the toxic factor. We needed to make clear whether it was fixed on to the erythrocyte stroma or whether it leaves the latter and goes into the supernatant fluid. With the aim of answering this question we performed a 10-minute centrifugation of the control and irradiated suspensions with a

subsequent aspiration of the supernatant in which the samples of ground tissue were then placed. The occurrence of characteristic "jumps" in the experimental curve indicates the passage of the toxic factor in the supernatant fluid (Fig. 5).

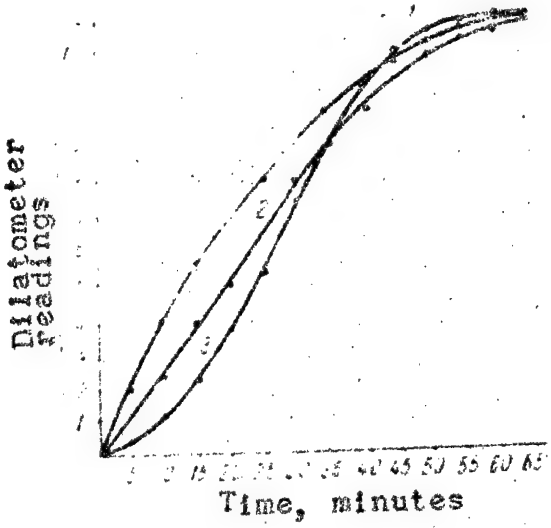

Fig. 4. Tissue Striction With Non-Radiation Hemolysis. 1 -- control; 2 -- hypotonic hemolysis; 3 -- saponin hemolysis.

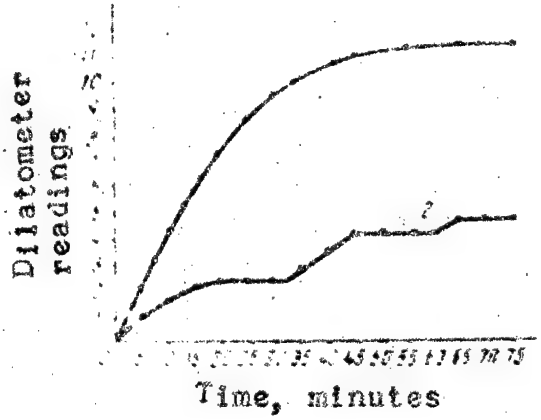

Fig. 5. Tissue Striction In Supernatant Fluid.
1 -- control without irradiation; 2 -- experiment after irradiation.

The nature of the toxic factor, naturally, continues to remain unclear. There are grounds for the belief that

it is in one way or another bound to the phospholipids or decomposition products of them. The lack of a toxic effect after hypotonic hemolysis (in which only the hemoglobin leaves the erythrocytes) as well as after saponin hemolysis, which is accompanied by the outflow of hemoglobin and cholesterol (D. L. Rubinshteyn and R. A. Rutberg) speaks for such a suggestion. Investigations of the electron-optic and dielectric structures of the irradiated erythrocytes, which show a considerable injury to them, can also serve as a supplementary confirmation of our surmise. The degeneration of a lipoprotein complex of red blood cells evidently underlies the latter (Yu. A. Kriger and Ye. S. Yelkhovskaya).

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Bone Changes in the Postnatal Period in the Offspring of Rabbits Exposed to the Effect of Ionizing Radiation at Different Periods of Gravidity

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Despite the considerable number of investigations directed at the study of the effect of ionizing radiation on bone tissue, this problem is the least studied. Certain authors (B. A. Arkhangel'skiy), describing the effect of radiant energy on the fetal cell and the fetus in rabbits, note the lag in the growth of fetuses irradiated in utero. Douglas observed a bone anomaly of the upper and lower extremities of a newborn child the mother of which had been given X-ray therapy in the fourth month of pregnancy. Similar anomalies and abnormalities have been noted by other authors (Murphy, Fas, Goldstein), which they observed after intrauterine irradiation of the offspring of mice. From works (Russell, Wilson, Schall, Basik) performed on mice, it is seen that a more harmful effect of irradiation is expressed on the offspring which are in the earlier phases of intrauterine development. The size of the dose of X-rays is also of importance: the damage is increased with the increase in the dose. N. A. Krayevskiy, in dealing with radiation sickness, notes microscopically demonstrable changes in the bone tissue of animals, which are observed chiefly in the periosteum, endostum of the vessels of the Haversian canals and osteoblasts in the growth zones.

The problem of our work was that of demonstrating possible changes in the osseous skeleton of the offspring of rabbits exposed in utero to a single X-irradiation at different periods of gravidity of the mother.

Total-body irradiation with a dose of 300 r was given under the following conditions: voltage 200 kv, current 15 ma, filter 0.5 mm Cu and 1 mm Al, distance 60 centimeters, half-value layer 0.95 mm Cu.

The rabbits were irradiated on the 13th and 20th days of gravidity, because at these period of embryonic development the so-called "critical phases of development" are observed in which the most pronounced changes may be expected. Thus, placentation in rabbits is completed by the 20th day. The period of greatest organogenesis occurs from the fifth through the 15th day (Russell). Over the course of the

19th day a marked increase is observed in the size of the embryo, which then becomes a fetus (G. A. Shmidt).

Young rabbits born of mothers which had not been exposed to X-irradiation served as controls.

By the method of roentgenography we studied the condition of the long bones of the extremities, directing attention to the times of appearance of the ossification centers chiefly for the diaphyseal and epiphyseal growth zones of the femoral and tibial bones in the area of the knee joint; the length of these bones was taken into consideration as was also the length of the trunk of the young rabbits together with the head.

X-ray films were taken directly after birth of the offspring as well as during the course of their growth. The technical conditions under which the films were made were kept the same and a constant skin-to-tube distance of 100 centimeters was used, which made the dimensions approximate the actual dimensions and made it possible to compare the figures obtained.

On histological preparations we studied the structure of the metaphyseal growth zones of the femoral bones pertaining to their distal segments. In addition to what has been stated, the weight of the experimental animals was noted. These data are presented in Tables 1 and 2; the numerical data in them have been treated by the method of variation statistics, making it possible to establish the average probable error for each magnitude and the reliability of the differences between each of two groups by comparison with the control groups of experimental animals.

In analyzing the data obtained it should be noted that the body weight and the length of the trunk (almong with the head) as well as of the long tubular bones of the rabbits irradiated in utero on the 13th and 20th days of their mother's gravidity were less than those of the control group and, in addition, were different from one another. Smaller values for all three indices were noted in the first group of experimental offspring.

The data of Table 2 show the delay in the appearance of the ossification centers for the epiphyses of the long bones in the area of the knee joints in the rabbit offspring irradiated with X-rays in utero on the 13th and 20th days of gravidity of the mother, whereby these changes were most pronounced in the first group by comparison with the second group of experimental animals and the control group. In part of the bunnies which we studied at different periods of their development within the limits of two weeks of life, the signs noted were maintained subsequently.

Table 1

Weight, Length of Trunk and Length of Various Long Bones of Young Rabbits on First Day of Life for Controls and Those Irradiated in Utero on the 13th and 20th Days of Gravidity of the Mother

Group	No of young bunnies	Weight g	Reliability of differences compared with control	Length of trunk plus head, cm.	Reliability of differences compared with control	Length of femur, mm.	Reliability of differences compared with control	Length of tibia, mm.	Reliability of differences compared with control	Length of humerus, mm.	Reliability of differences compared with control
Control	57	54±1,9	—	11±1,3	—	10,9±0,65	—	13,6±0,18	—	11,5±0,1	—
Bunnies irradiated on 13th day of gravidity of mother	39	40±1,3	5,0	9,7±0,3	3,2	9,9±0,35	2,5	11,4±0,16	9	10,5±0,1	7
Bunnies irradiated on 20th day of gravidity of mother	23	48,6±0,5	2,5	11±0,1	—	11,5±0,19	—	13,3±0,2	—	12,5±0,05	7

Table 2

Presence of Ossification Centers for Epiphyses of Long Bones of Extremities in Bunnies on the First Day of Life

Day of gravidity on which mother rabbit was irradiated	No of offspring bunnies examined	Presence of ossification centers for distal femoral epiphysis, %	Reliability of differences compared with control group	Presence of ossification centers of proximal tibial epiphysis, %	Reliability of differences compared with control group
13th	39	13±5	8,8	13±5	7,9
20th	23	52±10	1,6	52±10	1,6
Control group	57	75±6		72±6	

We were able to note that the absence of ossification centers on the first day of life of the bunnies irradiated in utero and then a delay in their appearance during the course of their life occurred also for the proximal femoral epiphyses, humerus and ulna, and for the distal epiphysis of the tibia, patella, as well as for the epiphyses of the metatarsal and metacarpal bones and phalanges of both extremities. This once again confirms the general disturbance in the growth of bones in length and in their formation. These disorders in bone development occurred chiefly in the rabbit offspring irradiated in utero during the first half of gravidity of the mother (on the 13th day of gravidity).

The distal segments of the metaphyseal areas of the femoral bones of the bunnies of the first day of life were taken for histological preparations directly after they had been killed. The bone tissue was fixed in 10-percent solution of formalin and then decalcified in five-percent nitric acid solution until softening occurred. After the bone was washed out, it was passed through alcohols of increasing strength. Sections were made after embedding celloidin. The preparations were stained chiefly with hematoxylin-eosin according to the van Giesson method.

The principal morphological changes amounted to a considerable reduction (impoverishment) in the blood supply in the growth zone areas. This was expressed in a thickening of the walls of the blood vessels, and in certain places a definite development of connective tissue could be noted crowding out the bone-marrow elements. In places, foci of hemorrhage were observed, and in localized areas, small elements with pycnotic changes in the nuclei and a destruction of the bone and marrow cells. The medullary trabeculae were swollen, and their structures were indistinct, and in places a thinning out of these trabeculae were seen. All these changes were noted in both groups of observations, but they were most pronounced in the preparations of bones from bunnies irradiated in utero on the 13th day of gravidity of the mother.

In addition to this, a certain difference could be detected also in the changes between the two groups of observations. In the offspring irradiated in utero on the 20th day of gravidity in the mother, there were incipient phases of changes, which were not particularly developed later. Thus, after irradiation on the 20th day of gravidity of the mother hyperemia and hemorrhage were noted in the growth zones of the bunny offspring, with hyperplasia and proliferation of the bone-marrow elements and the presence of a large number of young (beginning) forms rather than a

destruction of them. A swelling of the medullary trabeculae was also noted.

Conclusions

1. In the long bones of the extremities of the offspring of rabbits exposed to a single irradiation with X-rays at different period of gravidity of the mother disorders of osteogenesis, determined roentgenologically, are found in the postnatal period.

2. In histological preparations of the metaphyseal areas of the femoral bone obtained from bunnies irradiated in utero there are hyperplasia and proliferation of the bone marrow elements as well as a thickening and swelling of the medullary septa. Afterwards, a reduction occurs in the blood supply and the development of connective tissue occurs which crowds out the bone marrow elements; there is an indistinctness of the image on the film and a thinning out of the medullary trabeculae.

3. The X-ray and histological changes were more pronounced in the offspring of rabbits irradiated on the 13th day of gravidity than in the offspring of rabbits irradiated on the 20th day of gravidity.

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Early and Late Changes in the Skeletal Musculature of Rats Exposed to Local X-Irradiation

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There is exceedingly and limited contradictory information in the radiological literature concerning the biological effect of ionizing radiation on muscle tissue.

We made a study of traumatic regeneration of muscle tissue after irradiation.

The object of the investigation was the tibialis anticus muscle of adult white rats. In all, 80 animals were used in the experiment. The irradiation with X-ray was accomplished locally with a dose of 3,000 r (as the results of the preceding work showed, this dose was known to produce a disturbance in traumatic regeneration in the irradiated muscle). The leg and part of the thigh of the right extremity were exposed to the direct effect of X-rays. The body was screened with a three-millimeter lead plate.

Traumatic regeneration in the muscle was evoked as a response to mechanical injury inflicted with the blade of a safety razor at different time intervals after irradiation: after one day, two months, six months, one year after the irradiation. Recovery of the defect was studied in the third, fifth, 10th, 20th, and 30th day after the start of regeneration. The entire material was subjected to histological treatment.

The Effect of X-rays on the Intact Muscle. During the first few days after irradiation an inflammatory reaction is observed in the connective tissue of the endomysium. In the muscle tissue only certain reactive changes could be noted which were manifested in a disorientation of the nuclei and a reduction in the chromatin concentration in them. The sarcoplasm was unchanged. On the 15th-20th day definite signs of injury were found in the muscle tissue: waves of contraction, ruptures and thinning out of various muscle fibers. A focal proliferation of the cellular elements and homogenization of the collagen fibers, particularly around the blood vessels, was observed in the endomysium. After two months a thickening of the endomysium became noticeable between certain muscle fibers. The latter showed signs of partial atrophy independent of the connective-tissue changes.

Although the atrophic processes occur also the development of them during the first few months after irradiation proceeds extremely slowly. Six months after irradiation a shortening of the muscle investigated is found in part of the animals macroscopically. Eight months after irradiation definite signs of atrophy are found in the investigated muscle of all animals. The muscle is dense to the touch, and shortened by 0.5 centimeters in comparison with the normal. On microscopic examination a granular degeneration and muscle-fiber fragmentation are noted (Fig. 1). Sections of degenerating muscles are encapsulated in places. The intact muscle fibers are thin, have a tortuous course, are poor in contractile substance; at the same time, the myofibrils show a definite transverse striation. The muscle nuclei are disoriented along the course of the fiber, and the number of them is insignificant. The muscle fibers are divided by layers of connective tissue containing a considerable number of cellular elements. In the blood vessels a proliferation of endothelium and a proliferation of the adventitia are observed.

Fig. 1. Microphotograph. Fragmentation and Atrophy of the Muscle Fibers are Divided by Broad Bands of Connective Tissue Eight Months After Irradiation (Dose 3000 r). Zenker-Formol Fixation. Iron Hematoxylin Staining. Ocular 7x, Objective 40x.

In time the disturbances noted above progress so that a year after irradiation the atrophy of the muscle becomes even more pronounced. The muscle is shortened by 0.5-1.5 centimeters. The muscle fibers thin out to such an extent in places that their diameter does not exceed the diameter of ordinary muscle nuclei. The nuclei in such fibers are rarely found. Transverse striations become poorly discernible, and the muscle fibers themselves resemble interrupted narrow tortuous bands. In the layers of connective tissue the number of cellular elements is reduced. Collagen fibers are subjected to hyalinization. Endothelial proliferation leads to plugging up of the blood vessels.

In three out of 15 cases tumors of the fibrosarcoma type were found between the muscle fibers, starting from a

connective-tissue skin base. The tumor infiltrated the intramuscular connective-tissue bundles and compressed the muscle fibers to such an extent that the latter acquired the appearance of tortuous filaments.

The Effect of X-rays on Muscle Regeneration. In nonirradiated muscle a muscle-connective-tissue regenerate is found on the 30th day in the area of the defect with a predominance of muscle fibers. Differentiation of the latter achieves the state of the mature muscle fiber. Muscle regeneration proceeds from muscle buds growing out to the center of the wound from the ends of the damaged muscle fibers. In the irradiated muscle (3000 r) the recovery of the defect is delayed and is accomplished by connective tissue.

The cause of the change in the nature of regeneration in the irradiated muscle is an aggravation of the degenerative changes in the area of the injury, delayed phagocytosis, disturbance in normal fibroblastic reaction (inhibition of proliferative processes and rapid maturation of fibrillar structures) and the suppression of muscle tissue regeneration. Recovery of the latter in the control group begins on the fifth day, and in the experimental group the muscle buds are found only after the 10th day. At this time the wound begins to fill in with granulation connective tissue. The appearance of granulation connective tissue in the control contributes to a directed growth of muscle symplasts. Therefore, there are no grounds for the belief that young connective tissue in the experiment is a serious obstacle to the growth of young muscle elements. Nevertheless, the latter undergo regression. Only a few muscle symplasts are preserved up to the 30th day in the composition of the areolar connective-tissue regenerate filling in the muscle defect. The edges of the defect are subject to cicatrization.

The observation on the course of the regeneration process produced in the muscle 2, 6 and 12 months after irradiation showed that the effect of suppression of the regenerative capacity of muscle tissue is persistent (Fig. 2). Even a year after irradiation the regeneration of muscle tissue is suppressed and healing of the defect occurs chiefly through the connective tissue.

With time the effect of irradiation lessens in the muscle tissue. A tendency toward regeneration appears in it after 8 and 12 months. However, newly formed muscle elements (myosymplasts) do not reach final differentiation, because they are formed in a muscle which is involved in an active atrophic process. In the development of the latter a definite part is played by vascular disturbances and hya-

Fig. 2. Microphotograph. Thirtieth Day of Regeneration. Defect Filled in With Fatty Tissue. Traumatic Regeneration Produced in the Muscle Six Months After Irradiation (Dose 3000 r). Zenker-Formol Fixation. Staining with Karachi Hematoxylin and Eosin. Ocular 7 x, Objective 8x.

linosis of the connective tissue in thelate stages. Naturally, under these conditions the tendency toward regeneration of muscle tissue which occurs can not be realized, and the defect inflicted in the muscle is filled in with connective tissue a year after irradiation.

What is the cause of the changes observed in the muscle? On this question there are different opinions. Thus, Schmidt sees the cause of the changes occurring in muscle in an injury of the blood vessel wall; N. A. Dobrovol'skaya-Zavadskaya, Leach and Sigiura consider the morphological changes in muscle tissue the result of a direct effect of ionizing radiation. Apparently, both causes play a part, but at different times after irradiation one of them predominates. Undoubtedly, during the first few months after irradiation, when the increase in morphological changes occur extremely slowly in the connective tissue and in the blood vessels, the reactive and later degenerative changes in the muscle tissue are brought about chiefly by disturbances of the processes of regeneration in it. The basis for such a supposition is the fact that in the irradiated muscle the process of traumatic regeneration is markedly disturbed. At later periods, after six and particularly after eight months, when hyalinosis of the collagen fibers in the connective tissue, thickening of the wall and obliteration of the lumen of the blood vessels is seen to a progressively greater extent, the main cause of the muscle atrophy may be considered to consist specifically of these changes. They predominate and lead to a marked muscle atrophy, although at this time (after eight months and one year) the capacity for regeneration is regained in the muscle tissue itself. Experiments with traumatic regeneration produced a year after regeneration attest to this.

Conclusions

1. Local irradiation of the muscle with X-rays and

a dose of 3000 r produces serious disturbances in it. These disturbances are expressed in a depression of the regenerative capacity of muscle tissue and the development of muscle atrophy.

2. The effect of depression of the regenerative capacity of muscle tissue produced by irradiation is shown as early as the early periods after irradiation and is persistent. Only a year after irradiation does a pronounced tendency toward regeneration appear in the muscle tissue.

3. Atrophy of the irradiated muscle develops gradually and becomes distinct by the eighth month. A disturbance in the regeneration processes in the muscle tissue are of significance in the development of the atrophic process during the first few months after irradiation. At later periods (six to eight months) the connective-tissue and vascular disturbances play a definite part, in view of which the atrophic process increases rapidly.

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Treatment of Radiation Sickness Complicated by Traumatic Shock

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The problem of treatment of radiation sickness in cases of combined afflictions has been inadequately discussed in the literature. It has been stated only in individual works that the treatment of radiation sickness complicated by traumatic shock and burn trauma is carried out with the same agents which are used for the therapy of the pure form of radiation sickness with a similar beneficial therapeutic effect (N. S. Dzhavadyan, G. V. Sukyasyan, M. N. Novikova, A. L. Komendantova, Ye. A. Khrushcheva and others). Nevertheless, according to the observations of the majority of authors severe trauma, like, for example, fractures of the bones, injuries to abdominal organs, aggravates the course of radiation sickness (M. D. Abdulayev, N. I. Blinov, G. Sh. Vasadze, T. F. Ivanova, I. G. Isaakyan, A. G. Zemlyanoy and others). According to the data of A. L. Komendantova, Ye. A. Khrushcheva, A. P. Maysyuk and others, burn trauma of the first and second degree up to 10 percent of the body surface markedly aggravated the course of radiation sickness and led to a higher mortality rate of animals with combined afflictions by comparison with control animals.

The problem of present experimental investigation was the study of the course of radiation sickness complicated by mechanical trauma and the treatment of it.

There were three groups of animals in the experiment. The first two groups were controls, and in the third, comprehensive treatment of radiation sickness was carried out. The animals of all groups were subjected to X-irradiation in a dose of 700 r under the following conditions: voltage 185 kv, current 15 mm, filters 0.5 mm of Cu, skin-to-tube distance of 50 centimeters, dose rate 25 r/min. In the second group mechanical trauma was inflicted on the animals 30 minutes after irradiation -- 80 blows to the thigh muscles according to the Cannon method. In the third group following a similar combined effect the animals were subjected to comprehensive therapy for three weeks. At first, counter-

shock therapy was used and with this the treatment of radiation sickness was begun early (after two hours). The countershock combined therapy used one and two hours after the trauma of the irradiated animals consisted of the following agents: 1) dibazole in a 10 percent glucose solution; novocaine block and heating; 2) blood transfusion, glucose with vitamins and dimedrol [benadryl]. Using this combination it was possible to bring 100 percent of the rabbits out of shock with a mortality rate of 50 percent in the control experiments.

Taking into consideration the disturbances in the various functions in radiation sickness we used a combination of measures for the treatment of it consisting of substances which stimulate hematopoiesis, preventing the development of infectious processes, contributing to the removal of toxic products from the body, preventing the occurrence of hemorrhages, and substances regulating the metabolic processes.

This combination of agents included the following: repeated blood transfusions, repeated plasma infusion with a suspension of leucocytes and thrombocytes, repeated administration of vitamins (B_1, B_2, B_6, B_{12}, C, K, P), glucose, dimedrol and antibiotics. For the purpose of treating anemia reduced iron was used. Along with the therapeutic measures careful observation was made of the sick animals, and they were given a varied diet and regular care (a combination worked out by I. V. Il'yinskaya).

In all groups of experiments a study was made of the general condition of the animals, the length of life, changes in weight, body temperature, changes in the composition of the peripheral blood and bone marrow. All these indices were determined before the experiment and one, three, 24 hours, three, seven days and later, once a week for 63 days, after the irradiation and trauma.

In comparing the data obtained in the group with the combined afflictions and the results of experiments with ionizing radiation alone the course of radiation sickness showed no differences objectively: the general condition of the animals of both groups was depressed after the irradiation; they did not take food, and in part of the rabbits disorders in the gastro-intestinal tract functions developed promptly. The weight gradually decreased, and there was a marked exhaustion and anemia. There were no essential differences found either in the reduction in temperature in the groups compared. A difference was found with respect to the outcome of the sickness -- half of the rabbits exposed to irradiation alone suffered through the radiation

Name	Total experiments	Survived	Died	Mortality rate from shock, %	Mortality rate from radiation sickness, %
Radiation sickness (control)	8	4	4	-	50
Combined trauma	10	-	10	50	50
Combined trauma with treatment	10	9	1	-	10

sickness and survived, while in the group with the combined effect all the animals died, whereby 50 percent died from shock during the first day and 50 percent, from radiation sickness on the 7th-19th day. Therefore, the course of radiation sickness in combined afflictions and the outcome of it could be studied on only five rabbits. The therapeutic combination used made it possible to save the lives of 90 percent of the animals with radiation sickness complicated by trauma, whereas there was 100 percent mortality rate control experiments (see Table).

The course of radiation sickness in the majority of treated rabbits (third group) was less severe than in the animals of the first two groups. The weight of the treated animals decreased less sharply than the controls with combined affliction. The temperature ranged within limits of the original normal, and there were no gastro-intestinal disorders.

The hematological indices in the animals of both groups (control and with treatment) were considerably altered after combined afflictions. The results of the simultaneous investigation of the morphological composition of the bone marrow and peripheral blood showed a depression of the bone-marrow function reflected in the composition of the peripheral blood.

Changes in the red blood during the first period

of the disease were expressed slightly. Afterwards, an anemia developed which occurred sooner in the control animals (on the third to seventh day) than in the treated animals, and was more marked. In both groups anemia was associated with the depression in the processes of proliferation and maturation of the erythroblasts.

During the period of anemia aniso-poikilocytosis, hyperchromic macrocytes, basophilic strippling of the erythrocytes and certain other pathological forms were observed. Intestinal hemorrhages accelerated the development of anemia and led to the rapid death of the animals. In the group of treated animals anemia was observed only in three (out of 10) and occurred at a later time -- on the 14th-28th day.

Corresponding to these periods in the control animals a marked reduction in the reticulocyte count was observed up to a complete disappearance of them during the period of the climax of anemia and before death of the animals. In the treated rabbits following a period of reduction the reticulocytes increased in number and afterwards, throughout the entire observation, remained (with slight variations) within the limits of the original figures.

During the period from the third to the 14th day leucocytosis (which occurred after 24 hours) in groups with combined trauma in all animals was replaced by leukopenia with an absolute neutropenia and lymphopenia which was observed in control experiments until the death of the animals, which was associated with a depression of proliferation and maturation of the young cells of the leukoblastic series. In the group with uncomplicated radiation sickness no leukocytosis was observed after 24 hours. Similar results have been noted by N. S. Dzhavadyan, G. V. Sukyasyan and M. N. Novikova.

Along with the quantitative changes wualitative changes were noted throughout the radiation sickness: hypersegmentation of the neutrophils, fragmentation of the nuclei, vacuolization of the nuclei, degenerative monocytes, karyolysis and others.

In experiments with treatment the leucocyte count began to increase beginning with the 14th day, and by the 56th-63rd day approached the original figures. From the 14th-21st day the recovery of the bone-marrow hematopoiesis began in the majority of treated animals which was expressed in an increase in the erythro-, leuko-, and thrombopoietic functions and was reflected in the peripheral blood by an increase in the leucocyte, platelet and erythrocyte counts. In the surviving animals with pure radiation sick-

ness anemia remained for a longer time, and by the 63rd day the concentration of hemoglobin and erythrocyte count did not reach the original level.

Therefore, because of comprehensive therapy of radiation sickness carried out for 21 days the course of radiation sickness was less severe than in the control animals. Along with this, the degree of change in all the hematological indices in the group of treated animals was less pronounced than in the control animals, and by the 63rd day analyses of the peripheral blood and bone marrow did not show any essential deviations from normal. Regular treatment for three weeks was very important, of which we were convinced in two experiments (Nos 28 and 29) in which treatment of the rabbits was stopped early when they were in a good general condition. In connection with this, the rabbits died after two or three days.

Conclusions

1. Trauma inflicted on irradiated (with a dose of 700 r) animals aggravated the course of radiation sickness and led to the death of all the rabbits; nevertheless, after a single irradiation with the same dose the death of the animals amounted to 50 percent, and after trauma, to 30 pecent.

2. Comprehensive treatment of radiation sickness in animals which had been brought out of a state of shock (blood transfusion, plasma infusion with leucocytes, administration of glucose with vitamins, dimedrol, injections of antibiotics, oral administration of synthomycin [chloramphenicol], iron, vitamins for 21 days) saved the lives of the great majority of animals.

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The Use of Vitamin B_{12} and B_6 Under Conditions of the Repeated Effect of X-rays

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The development of methods of prophylaxis and therapy of chronic and subacute radiation afflictions, particularly hematopoietic disturbances, is acquiring great importance at present. Information in the literature is sparse in this direction.

The aim of our investigation was the study of the efficacy of vitamins B_{12} and B_6 in subacute disorders of hematopoiesis produced by repeated irradiations with X-rays. In the literature there is mention of the favorable effect of liver preparations, vitamin B_{12} and vitamin B_6 in radiation sickness as complications of X-ray therapy. In experiments with a single irradiation of rats and dogs (M. S. Lapteva-Popova), rabbits (Covazzutti, Dagnini) and guinea pigs (Mücce, Morczek, Werner) a reduction in the hematopoietic changes was noted under the influence of therapeutic and particularly the prophylactic use of vitamin B_{12}. Carter, Busch and Strang injected vitamin B_{12} into rats irradiated with a dose of 400 r, and did not note any favorable effect on bone marrow or peripheral blood.

We did not find any data in the literature on the use of vitamin B_{12} in repeated irradiations. With radioactive phosphorus intoxication of rats vitamin B_{12} exerted a beneficial effect only with the $LD_{50/30}$. There are several works on the use of vitamin B_6 (pyridoxin) (Lawrence, Scott and others, Haltern; Reeves; Oppenheim and others); however, they all deal with the effect of vitamin B_6 on the course of radiation sickness in patients who had been exposed to local radiation therapy, and they do not contain information concerning the condition of hematopoiesis.

The work was carried out on 35 dogs -- males and females -- of different weights (8-18 kilograms) which were subjected to a daily irradiation on an X-ray therapeutic RUM-3 apparatus. The irradiation conditions were the following: voltage 180 kv, current strength 15 ma, filters 0.5 mm of Cu and one mm Al; dose rate 2.4 r/min., single dose of 20 r, total dose of 500 r in 25 sessions. The clinical state of the animals, the changes in the morphological indices of the peripheral blood and in the bone marrow smears were investigated.

In the first series of experiments six dogs were given B_{12} intramuscularly in a dose of 15 gamma for the en-

tire period of irradiation at intervals of three to four days (total 120 gamma). Along with this, after each irradiation the dogs were injected intravenously with 10 cubic centimeters of a 40-percent glucose solution containing 250 milligrams of ascorbic acid. One of the dogs died on the second day after the radiation effect was stopped (after obtaining a total dose of 500 r) with signs of a marked hemorrhagic syndrome.

In the other dogs the clinical picture of the disease was expressed only during the period of irradiation; the white blood count in them decreased gradually, reaching a minimum of 32 percent of the original a week after stopping the irradiation, whereas in the control dogs the white blood count dropped to 24 percent of the original.

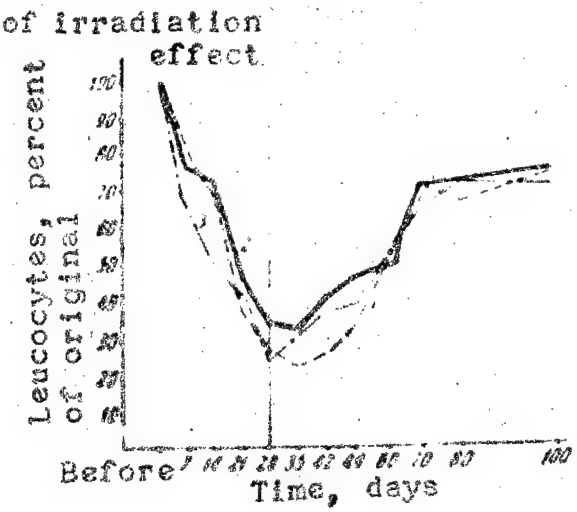

Fig. 1. Change in the White Blood Count of Control Dogs (1), Dogs Given Vitamin B_{12} at Intervals of Three or Four Days (2), and Dogs Given Vitamin B_{12} Daily in Combination with Vitamin B_6 (3).

(The white blood count is given in percentages of the original.)

The recovery in the white blood count of the treated dogs began earlier than in the control dogs, the counts were higher during the period up to 60 days (Fig. 1). By the 70th day the white blood count reached 71 percent and varied within limits of 71-74 percent during the subsequent observation up to the 150th day. No differences were observed during this period between the leucocyte counts of the treated and control dogs.

The red blood count of the treated dogs of this

series dropped to a greater extent than the control animals by the 42nd-49th day (77 percent in the experiment and 87 percent in the control). The changes in the reticulocyte count were the same. Bone marrow smears of treated animals were richer in cellular elements and contained cells in the early stages of erythro-myelopoiesis at almost all periods of the investigation in a somewhat larger quantity than in the control animals. However, the recovery in the red blood count of the treated dogs occurred somewhat more slowly (by the 80th day) than in the control dogs (by the 60th day).

In order to clarify whether the daily intravenous infusions of glucose with ascorbic acid exerted a harmful influence on the concentration of erythrocytes in the peripheral blood we performed a control experiment. Three dogs were subjected to a daily irradiation under standard conditions, receiving intravenously 10 cubic centimeters of a 40-percent solution of glucose and 250 milligrams of ascorbic acid. Prolonged observation of these animals did not show any considerable difference in the hematopoietic indices compared with the control.

The red blood count decreased to the same degree as in the control dogs.

In the second series of investigations three dogs were injected with vitamin B_{12} daily in a quantity of 15 γ for the first two weeks of irradiation; in the second two weeks five times a week, and when the irradiation was stopped, for another two weeks three times a week. During the experiment the dogs were given 405 γ of vitamin B_{12}.

The white blood count of the treated dogs of this series was notably higher than in the control animals. Beginning with the 56th day of the experiment the recovery in the white blood count of the treated and control dogs occurred at the same rate.

The platelet count of the experimental dogs decreased somewhat more slowly than in the control dogs. The recovery began earlier; however, by the 80th day of the experiment the difference in the number of platelets in the treated and control dogs disappeared.

The red blood count a week after beginning the irradiation increased by 10, 15 and 17 percent. In the bone marrow at this time and against a background of impoverishment of cellular elements a slight, relative increase in the number of erythroblastics cells was noted, a reduction in the concentration of young forms among them and an increase in the number of mature forms. Therefore, an acceleration of erythroblast maturations occurred.

At the time of receiving the total dose of 500 r (28th day) the erythrocyte count in the treated dogs dropped to a greater extent than in the control dogs (in the experiment, 77-85 percent; in the control, 93 percent); however, by the 60th day the composition of the myelogram of the treated dogs was close to the original. Normalization of erythropoiesis of the control dogs began later and was not so stable.

In the third series of investigations we decided to use vitamin B_6 (pyridoxin) along with vitamin B_{12}; the former plays an exceptionally important part in the metabolic processes, particularly in the transformations of nitrogenous substances. Five dogs were given vitamin B_{12} according to the system used in the previous series. At the same time, over the course of six days before the irradiation and the first two weeks of the irradiation period three dogs out of five were given vitamin B_6 in a quantity of 50 milligrams per os; the other two, 25 milligrams each intravenously.

The results of the observation showed that the hematopoietic changes of all five dogs given vitamin B_6 both by mouth and intravenously were the same. The white blood count decreased to the same degree as in the controls, but regeneration during the period between the 35th and 49th day occurred somewhat more rapidly (see Fig. 1). Thereby, in the majority of dogs the recovery in the neutrophil count occurred more rapidly by comparison with the recovery in the number of lymphocytes.

The platelet count of the experimental dogs changed in the same way as in the control animals, except on the 49th and 56th day there were higher figures in the treated animals. The erythrocyte count in two dogs of this series was maintained within limits of the previous normal for the entire period of irradiation, while in three dogs it decreased by no more than 15-16 percent. There were no hemolyzed erythrocytes encountered in the peripheral blood smears. The reticulocyte count decreased to a lesser degree than in the other groups.

No impoverishment of cellular elements was observed in the bone marrow of these dogs. The content of the cells of early generations of both hematopoietic series was high for the entire experimental period, and the number of mitoses of the red series decreased to a lesser degree. A considerable increase occurred in the content of erythroblastic elements of the bone marrow, apparently not only of a relative but also of an absolute nature. At the same time, a depression of myelopoiesis was observed which was expressed in a reduction in the total quantity of myeloid elements,

the appearance of degenerative changes in the cells of the myeloid series; however, these signs were brief, and normalization of hematopoiesis occurred sooner than in the control dogs (Fig. 2).

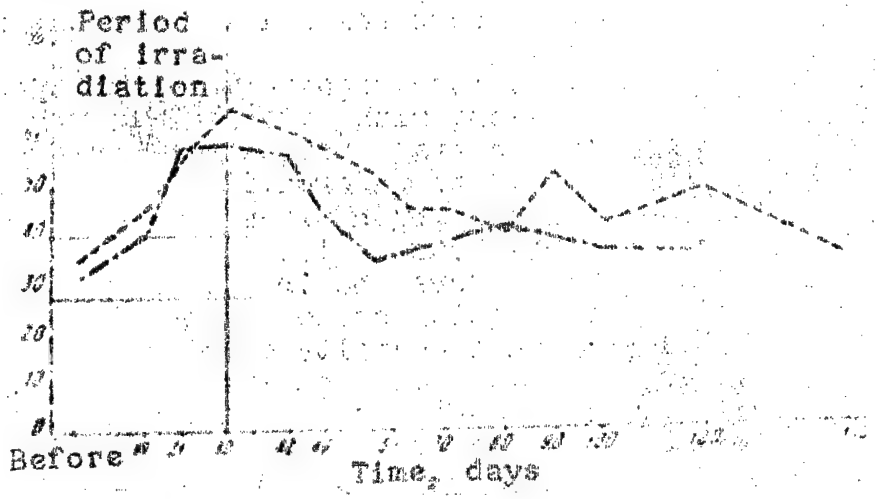

Fig. 2. Change in the Total Number of Erythroblastic Cells in the Sternal Bone Marrow Smears of Dogs Which were Given Vitamins B_{12} and B_6 (1) Daily During the Irradiation, by Comparison With the Control Animals (2).

Therefore, the combined use of vitamins B_6 and B_{12} proved to be very effective, particularly with regard to erythropoiesis.

For the purpose of clarifying the effect of vitamin B_6 in a quantity of 50 milligrams per os for six days before irradiation and during the first two weeks of irradiation. The leucocyte count and the platelet count of these dogs during the irradiation period decreased in the same way as in the control dogs, but regeneration began earlier, and during the period from the 49th to the 80th day the content of them was higher in the treated dogs. The red blood count decreased earlier than in the control animals, reaching a minimum level by the 28th day. The degree of reduction in the red blood count in the treated and control dogs was the same. Recovery in the treated dogs began earlier than in the control dogs, and by the 50th day the original level was reached, whereas in the control dogs the red blood count approached the original on the 60th day.

The material presented shows that the use of vitamins B_{12} and B_6 does not prevent hematopietic disorders occurring in repeatedly irradiated dogs. In treated dogs a certain

impoverishment of the bone marrow in cellular elements develops also, chiefly of the white series. A number of degenerative changes occur in the myeloid cells; however, these phenomena are somewhat less pronounced than in the control animals. The leucocyte count and the platelet count decreased during the irradiation period in the same way as in the control dogs.

In experiments with the daily use of vitamin B_{12} beginning with the first week of the irradiation, an acceleration of erythroblast maturation was noted which led to an increase in the red blood count in the peripheral blood by the end of the first week of the experiment. The use of vitamin B_{12} at intervals of three to four days also contributed to an acceleration of erythroblast maturation (by comparison with the control), but less considerable than after the daily administration of vitamin B_{12}. The entrance of mature erythroblastic elements into the peripheral blood against the background of inhibition of erythropoiesis leads to a depletion of the bone marrow of the dogs. This apparently can explain the earlier reduction in the erythrocyte count of dogs which have been given vitamin B_{12} by comparison with the control animals.

The cause of the greater reduction in the erythrocyte concentration in dogs given vitamin B_{12} at intervals of three or four days along with the daily infusions of glucose with ascorbic acid is hard to explain. Possibly the hemolysis of erythrocytes, which was observed in the peripheral blood in the second to third week of the irradiation, was of importance. Nor has the possibility been excluded of the significance of individual reactivity of the animals. It may be said with confidence that the regenerative processes of the animals given vitamins B_{12} and B_6 were depressed to a lesser degree than in the control animals. The less severe reduction in the number of young elements of the white and particularly of the red series as well as the earlier onset of recovery in the leucocyte and platelet counts are evidence of this. During the course of two or three weeks after the cessation of the irradiation the white blood count and platelet count in the treated dogs were higher than in the control dogs. However, by the 50th-60th day of the experiment the difference in the indices between the treated and control dogs disappeared, which may be explained by the cessation of vitamin administration.

The best results were obtained from the use of vitamin B_{12} in combination with vitamin B_6. The bone marrow in these animals was rich in cellular elements throughout the

entire period of observation, the cells of early generations were more numerous than in the other animals. A reduction in the erythrocyte concentration did not occur in all dogs and was considerably less pronounced.

The mechanism of the therapeutic effect of vitamin B_{12} was unclear. The opinion is generally accepted that it represents the extrinsic antianemic factor, which after combination with the intrinsic factor -- gastromucoprotein -- is deposited in the liver, and from there enters the bone marrow, regulating the hematopoietic processes. The possibility of the effect of vitamin B_{12} through the nervous system has not been excluded: favorable results obtained by a number of authors from the use of vitamin B_{12} in neurological manifestations of pernicious anemia, diabetes, as well as in the early period of polyneuritis (Scott, Sancetta and Ayres, 1950; Lereboulet and Pluvinage, 1951) point to this. On behalf of the nerve-reflex effect of vitamin B_{12} are the data of Ross, Mollin, Cox and Ungley, who observed a favorable effect of the vitamin introduced per rectum, where the absorption is negligible. As far as vitamin B_6 is concerned, as is well known from the works of A. Ye. Braunshteyn and his co-workers, it is an irreplaceable factor participating in the processes of synthesis and assimilation of protein. These qualities suggest its efficacy in such pathological conditions in which the acceleration of regeneration processes is necessary. Apparently, the efficacy of vitamin B_6 would have been increased if it had been used longer before irradiation, because in this case it might have exerted a more pronounced effect on metabolic processes.

Conclusions

1. The use of vitamins B_{12} and B_6 under conditions of prolonged daily irradiation does not prevent hematopoietic disorders. However, the inhibition of hematopoiesis, particularly erythropoiesis, in dogs given vitamins B_{12} and B_6 is less pronounced.

2. In dogs treated with vitamins a tendency is observed toward an earlier normalization of the relationship between the erythroblastic and myeloid elements in the bone marrow by comparison with controls.

3. In the peripheral blood of dogs given vitamins B_{12} and B_6 the content of white blood cells and platelets during the two or three weeks after stopping the radiation effect was higher by comparison with the control animals.

4. The combined use of vitamins B_{12} and B_6 was most

effective. The bone marrow of the animals was rich in cellular elements throughout the entire observation period and contained a large number of regenerative forms of the white and particularly of the red series.

5. The use of vitamin B_{12} alone during the initial period of irradiation produces an acceleration of erythroblastic maturation in the bone marrow, which involves the development of earlier erythrocytopenia in treated animals by comparison with the controls.

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Change in Sensitivity to Visceral Trauma of Animals Which Have Suffered From Radiation Sickness

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The change in the reactivity of an irradiated organism is of special importance in the pathogenesis of radiation sickness, because after the irradiation a limitation of the protective reactions is noted in response to various environmental influences (P. D. Gorizontov). Many research workers have noted that under the influence of trauma radiation sickness develops in a more severe form; death occurs from a smaller dose of ionizing radiation and more rapidly with combined afflictions (Brooks and others; Baxter and others; A. I. Morozov).

According to the data of A. I. Reshetov, irradiated rabbits in the initial period of radiation sickness (dose 650 r) are most resistant to trauma produced by stimulation of the sciatic nerve with an alternating current in combination with insignificant blood loss. The state of least resistance is noted in rabbits during the period of resolution of radiation sickness, and in dogs, during the period of the climax of it. G. M. Gorban' and P. P. Saksonov noted that the animals in which surgical procedures, particularly extremity amputations, were performed during the period of the climax of radiation sickness died, as a rule, 20-30 hours after the operation. V. K. Kalugin observed an increase in the resistance of experimental animals to mechanical trauma during the latent period of radiation sickness; here, shock occurred after considerable trauma, and the life span of the animals was increased somewhat by comparison with the controls.

Ye. N. Antipenko, A. M. Mgebrov and N. P. Sinyakina reported that by the method of producing interference between the food and defense reflexes functions which have already become stabilized following clinical recovery may be decompensated, and certain symptoms of radiation sickness may be reproduced. A change in the functions was maintained for two or three weeks after the interference between the reflexes. According to the data of V. N. Pravetskiy, dogs which have had radiation sickness did not tolerate bleeding so well in the period immediately following clinical re-

covery, and approximately a year later their sensitivity to blood loss was about the same as that of the control animals. The sensitivity to operative trauma at the climax of radiation sickness is increased. Therefore, themajority of investigators recommends performing surgical procedures in the latent period of radiation sickness, which provides a favorable course of the post-operative period before the development of signs of radiation sickness (L. M. Kotel'nikov; I. Ya. Tikhonin and others, A. V. Tsagareyshvili and I. I. Dorokhov; Gustafson and Cebul).

We did not find any data concerning the sensitivity of the body to visceral trauma at later periods following irradiation in the literature available to us.

The aim of the present work was a study of the characteristics of development of operative shock in experimental animals which have radiation sickness at various intervals after clinical recovery.

The experiments were performed on rabbits which had been subjected to a total-body X-irradiation with a dose of 500-800 r. The irradiation conditions were the following: RUM-3 apparatus, voltage 180 kv, current 20 ma, filters 0.5 mm Cu and 0.5 mm Al, dose rate 18 r/min., skin-to-tube distance 60 centimeters. For the experiments with experimental operative shock animals were used in which clinical recovery had occurred (normalization of the blood picture, recovery of the normal weight). The general condition and behavior of the animals which had had radiation sickness were outwardly no different from those of the non-irradiated animals.

For the purpose of studying visceral trauma the rabbits were tied down to a table in a supine position. The blood pressure was recorded with a mercurial manometer from the carotid artery on a smoked kymograph strip. The record of respiration was made by means of a cuff and a Marey capsule. The body temperature was taken rectally using a microelectrothermometer MT-55. For the purpose of evaluating the functional state of the vasomotor center a study was made of the pressor and depressor vascular reflexes. The pressor reflex was produced by compression of one carotid artery (where the other artery was tied off); the depressor reflex, by means of stimulating the centripetal segment of the depressor nerve with an induction current. The reflexes were studied in response to two stimulation intersities -- weak and strong, which it made it possible to judge the nature of the strength relationships of these reflexes during the course of development of shock. In the study of the pressor reflex compression of the artery for five seconds

was used as a weak stimulus; compression for 15 seconds, as a strong stimulus.

After obtaining the initial data the abdominal cavity was incised by a midline incision; the small intestine was extracted, and a mechanical stimulation of it was performed by means of traction; each evisceration lasted two minutes with an interval between them of 20 minutes during the course of which the indices under study were investigated. The infliction of trauma was continued until the development of shock. The occurrence of it was characterized by a pronounced and prolonged reduction in blood pressure, decrease in the body temperature, development of cardiac insufficiency, and a disturbance in the respiratory rhythm, as well as changes in the magnitude of the vascular reflexes and a development of phasic states in them.

A total of 33 experiments were performed; of these, 14 were on control and 19 on animals which had had radiation sickness. The animals which recovered were divided into two series. In the first series, which included 11 animals, the acute experiments were performed two or three months after irradiation. The animals of the second series (eight rabbits) were used in the experiment eight to nine months after irradiation.

As the results of the experiments showed, the resistance to visceral trauma in the animals which had had radiation sickness was considerably different from the resistance of control healthy rabbits. The degree of change in the resistance depended considerably on the time which had elapsed after the irradiation.

As seen from Table 1, in the control group of experiments the traumatic chock developed, on the average, after the sixth evisceration; here, the blood pressure dropped from 114 to 50.5 millimeters of mercury (by 56 percent). The most pronounced decrease in blood pressure was observed after the first few evisceerations; thus, after three eviscerations it dropped by 39 percent, whereas the next three eviscerations produced a reduction of only 17 percent.

Table 1

Change of Resistance to Visceral Trauma (Average Data By Series)

Series	Development of shock		Change in blood pressure			Change in body temperature			Length of life in state of shock
	No of evi-cerations	Time from beginning of trauma	Original (in mm of Hg)	In state of shock (in mm of Hg)	% of original	Original	At end of experiment	Difference	
First -- 2-3 months after irradiation . .	2	42 min.	116,5	41,2	35,4	38,1	32,6	—5,7	4 hr. 27 min.
Second -- 8-9 months after irradiation . .	6	1 hr. 59 min.	113,6	44,2	39	38,2	30,7	—7,5	3 hr. 26 min.
Control. . .	6	2 hr. 12 min.	114	50,5	44,2	38,0	32,1	—5,9	10 hr. 32 min.

Table 2

Number of Eviscerations Needed for the Development of Shock in Irradiated and Non-irradiated Animals

Series	No of experiments in series	Arithmetic mean according to series	Mode		Extreme deviations			
			No of evicerations	No of experiments	Least	No of experiments	Greatest	No of experiments
First -- 2-3 months after irradiation	11	2	2—3	7	1	3	5	1
Second -- 8-9 months after irradiation	8	6	4—5	6	4	2	10	1
Control	14	6	7—8	5	2	2	9	2

The considerable variations in the individual resistance of the rabbits to visceral trauma deserve attention: in some experiments shock developed after two eviscerations (in two experiments out of 14, see Table 2), in others, after nine (in two experiments of this group). The pulse rate of the control animals changed within broad limits. However, with the infliction of visceral trauma a gradual slowing of the pulse was observed (on the average, from 210 to 174 at the end of the experiments). The range of variations in the respiratory rate was just as broad. On the average, before the infliction of trauma it amounted to 56 per minute; at the end of the experiments, 50 respirations per minute. With the infliction of trauma hypothermia developed. At the end of the experiments the average reduction in body temperature was equal to 5.9°. The most intense drop in body temperature was observed after laparotomy and the first two or three eviscerations. The degree of reduction in the temperature depends to a considerable degree on the number of intestinal eviscerations performed, that is, on the duration of time in which cooling of the body occurs, which is associated with a more intense heat loss during the time that the abdominal cavity is exposed.

A very important index of the development of shock is the change in the vascular reflexes. In almost all experiments the absolute magnitude of them decreased with the infliction of trauma. With the deterioration in the condition of the animal more deep-seated parabiotic phases were noted up to complete inhibition of these reflexes.

The changes in the vegetative functions of the control animals occurred, on the average, after two hours and 12 minutes. The rabbits by this time were markedly inhibited and were in a state of profound torpid shock. The length of life in the state of shock varied within broad limits (from one hour to 24 hours).

In animals which had had radiation sickness the sensitivity to visceral trauma depended on the time which had elapsed after irradiation. From Table 1 it is seen that rabbits which had suffered from radiation sickness were particularly sensitive to trauma three months after irradiation. In order to produce shock in these animals it was enough to perform two intestinal eviscerations (average data for the group). The blood pressure dropped, on the average, by 64.6 percent in contrast to the control experiments, in which temporary rises in the blood pressure were observed not uncommonly during the course of infliction of trauma, in the irradiated animals the reduction in blood pressure was persistent, and there was no temporary normalization in the hemodynamics.

Considerable individual variations in the resistance of the animals to trauma, particularly in the control experiments, made necessary a statistical analysis of the results obtained. For this purpose, the arithmetic mean number of eviscerations was determined which was necessary for the development of shock, the mode, that is, the most frequently encountered variant, and the extreme deviations from the mode (Table 2).

From Table 2 it is seen that the resistance to trauma, judging by the number of eviscerations necessary for the development of shock, is different in different series. The difference between the first and the control series is distinctly seen. As has been noted above, shock in the first series developed, on the average, after two or three eviscerations of the intestine, and only in one experiment were five eviscerations performed.

In the second series of experiments four to five eviscerations were required, and in the control experiment the mode was equal to seven to eight eviscerations. In the irradiated rabbits a lessening of the individual reactions to trauma was noted, which was expressed in an approximation of the arithmetic mean to mode and to the least deviation in the extreme variants from the average value. In the rabbits the resistance to trauma eight to nine months after irradiation was less than in the non-irradiated rabbits, judging by the mode (which, in our opinion, is more reliable than the evaluation made according to the arithmetic mean).

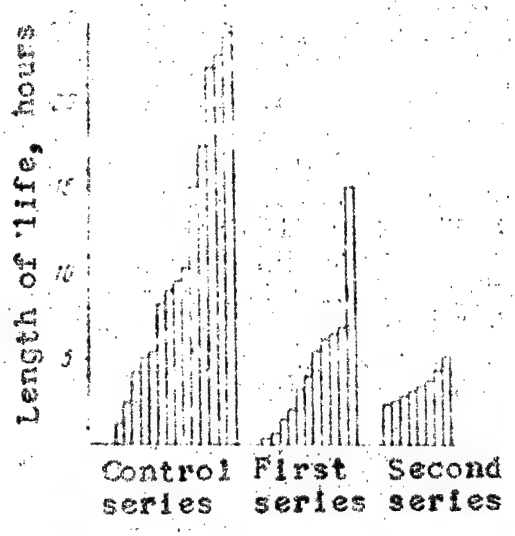

Fig. 1. Length of Life of Rabbits in A State of Shock

More pronounced differences between these series were noted in the length of life. It proved to be three times less in animals of the second series than in the control animals, and was lower by comparison with the length of life of rabbits in the first series. In Fig. 1 it is distinctly seen that in the state of shock the length of life of the animals decreased in both groups, particularly in rabbits which had been subjected to trauma eight to nine months after irradiation. In Fig. 1 a lessening of the individual differences in the life span of animals of the second series is also seen.

There were no clear-cut differences between the control and experimental groups in the change in respiratory and cardiac frequency. It needs to be noted only that in the rabbits which had had radiation sickness individual "intercalated" deep inspirations were found more frequently (in five out of 11 experiments in the first series and two out of 14 control experiments); this is an index of the deep-seated changes in the respiratory center (I. R. Petrov).

The average reduction in body temperature of control animals was somewhat greater than shock in the first group developed much more rapidly it becomes clear that the degree of reduction in body temperature of animals which had had radiation sickness was greater than in the control animals.

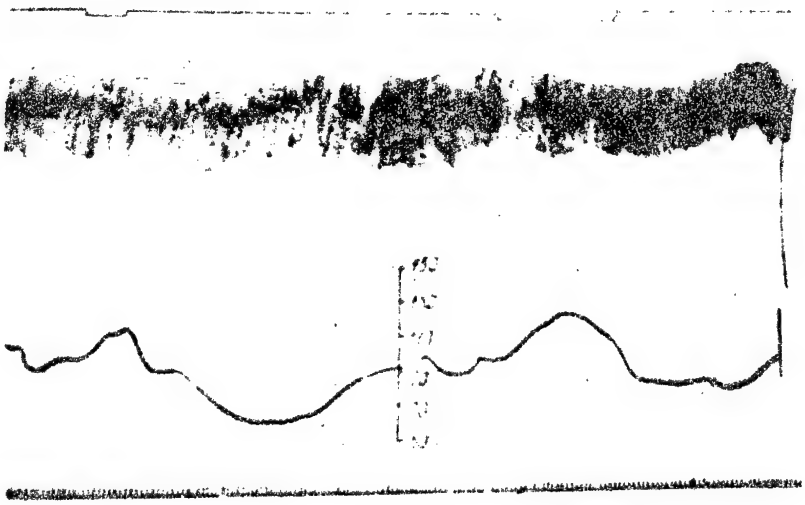

Fig. 2. Pressor Carotid Sinus Reflexes In the Original Condition in a Rabbit of the First Series.

On the left -- reflex to weak stimulus; on the right, to strong stimulus. From above down: record of reflexes, respiration, blood pressure, record of time (one-second intervals).

The reduction in the absolute magnitude of the reflexes and a disturbance in the normal strength relationships with the infliction of trauma and the development of shock was a general characteristic of the change in the vascular reflexes. In the animals which had had radiation sickness the change in vascular reflexes was characterized by a number of features. A more rapid reduction in the absolute magnitude of the vascular reflexes was observed in them. A more pronounced increase in the latent period of

the reflex was noted (by two or three times compared with the control experiment). In certain experiments of the first series a biphasic reaction of the pressor vascular reflexes was observed even in the original condition, that is, in response to compression of the carotid artery a pressor-depressor wave of the reflex appeared; thereby, the depressor phase was greater than the pressor (Fig. 2). Very frequently (in seven of 11 experiments of the first series) stimulation of the depressor nerve was accompanied by a prolonged after-effect characterized by a slow recovery of the original level of blood pressure (Fig. 3), whereas in the control experiments such a reaction was noted in the state of definite shock after the fifth to seventh intestinal evisceration.

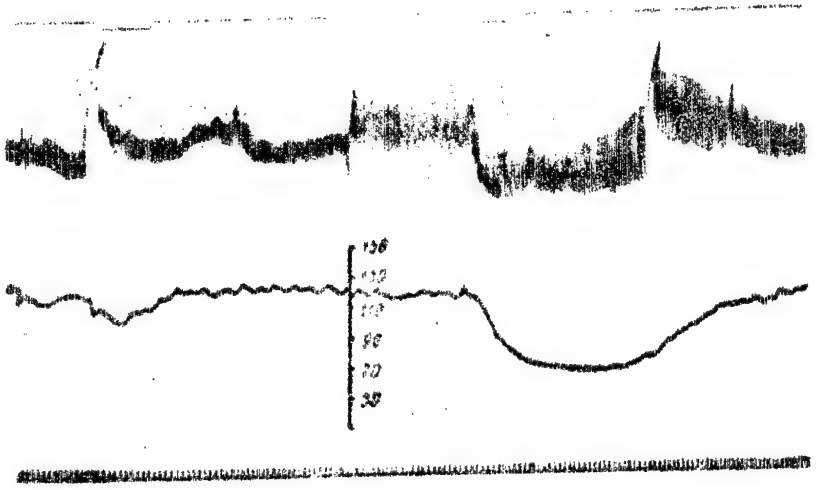

Fig. 3. Depressor Reflexes in the Original State of Rabbit In the First Series.

On the left -- reflex to weak stimulus; on the right -- to strong stimulus. Significance of the curves is the same as for Fig. 2.

As far as the experiments of the second group are concerned, the early inhibition of vascular reflexes was more characteristic of them than of the controls. In Fig. 4 kymograms are presented of the experiment indicating a change in the depressor vascular reflexes in the course of development of shock (experiment No 7 eight months after irradiation). In the original condition the vascular reflexes show normal strength relationships (the magnitude of the reflex to a weak stimulus is 16 millimeters; to a strong stimulus, 42 millimeters). After the first evisceration an equalizing phase of depressor reflexes occurred (magnitude

of the reflex 28 millimeters to a weak stimulus, and 30 millimeters in response to a strong stimulus). After the third evisceration the blood pressure increased somewhat, but an anesthetic phase occurred, that is, the reflex disappeared in response to the weak stimulus and decreased in response to the strong stimulus. After the fourth evisceration an inhibitory phase developed. The fact deserves attention that all these changes in the reflexes occurred with a relatively high blood pressure level. The rabbit died three hours and 17 minutes after the fourth evisceration.

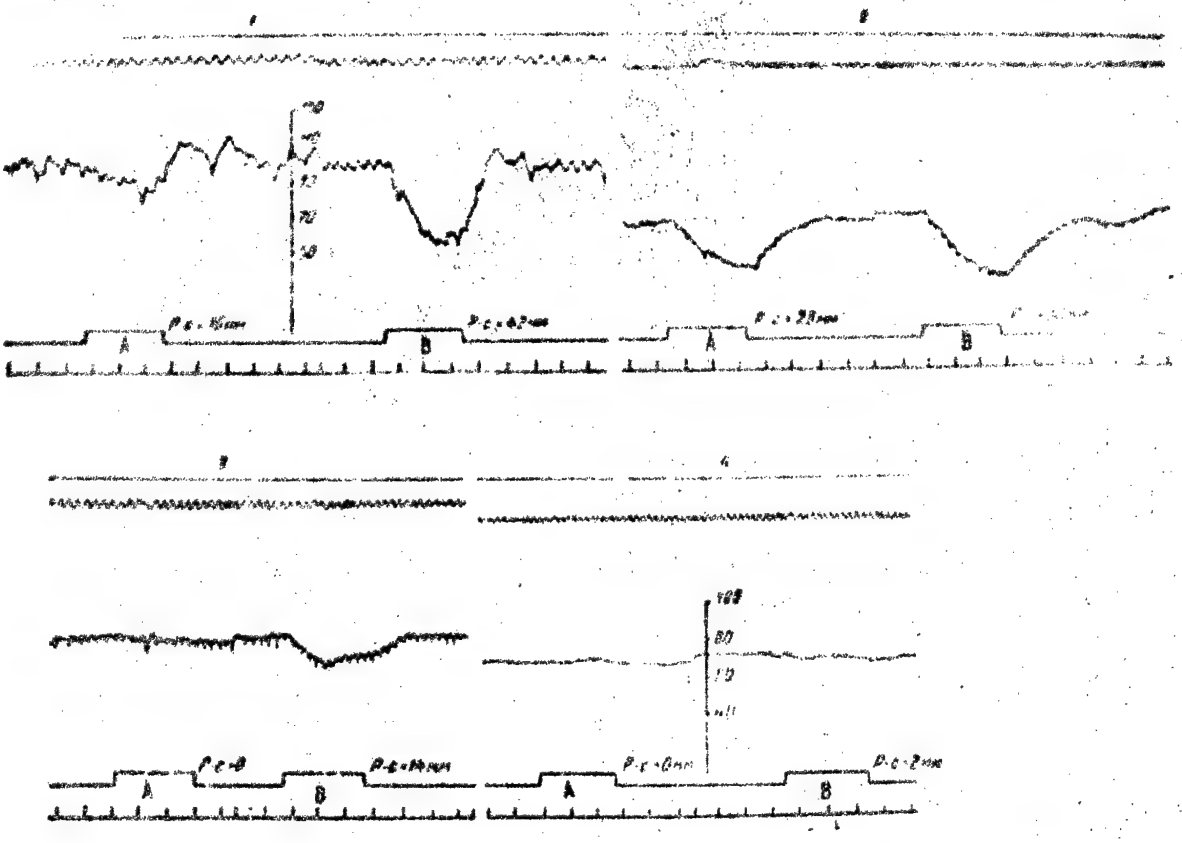

Fig. 4. Change in Depressor Reflexes During the Course of Development of Traumatic Shock in Rabbit of the Second Series.

1 -- reflexes in original condition; 2 -- reflexes after the first intestinal evisceration, development of equalizing phase; 3 -- reflexes after third intestinal evisceration, development of anesthetic phase; 4 -- reflexes after fourth evisceration, development of inhibitory phase; A -- reflex in response to weak stimulus; B -- reflex in response to strong stimulus. From above down: respiration, blood pressure in millimeters of mercury, record of the

evisceration effect, record of time (five-second intervals).

From the experiment it is seen that in the rabbits which had had radiation sickness the disturbance in the reflex regulation of circulation and respiration after infliction of visceral trauma was manifested considerably earlier than in the non-irradiated animals, and was of a more profound nature.

Therefore, rabbits which had had radiation sickness were sensitive to visceral trauma to different degrees, and their life spans after trauma were considerably shorter than in the control animals. The period of two to three months after irradiation was insufficient for the recovery of the usual resistance of the rabbits to trauma. This may be explained by the developing functional disturbances, which were not expressed clinically. Resistance to visceral trauma was reduced even eight to nine months after irradiation. Although just as many eviscerations were required, on the average, for the development of shock in these rabbits as in the control experiments their life spans after trauma were three times less. At the same time, a lessening of the individual variations in resistance to visceral trauma was noted.

An analysis of the changes in reflex regulation of the circulation showed that in the animals which had had radiation sickness it was inadequately compensated. A disturbance in the strength relationships of the vascular reflexes was shown even in the original condition, before the infliction of visceral trauma. An inertia of the nerve processes was observed, which was expressed in the lengthening of the latent period of the reflexes and a prolonged aftereffect. A more rapid inhibition of vascular reflexes occurred during the course of development of shock. In animals which had had radiation sickness it was sufficient to exert some slight external effect on them in order to reveal the imperfect nature of the reflex regulation of the circulation. Evidently, this was a manifestation of the limitation of the protective mechanisms, which in their turn are determined by the functional changes in the neurohumoral regulation of the irradiated organism.

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Doses of X-Radiation to Which Patients and Medical Personnel are Subjected During Cardiac Catheterization

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Despite the considerable degree to which the method of cardiac catheterization under the control of X-rays has become widespread, it has been inadequately studied from the hygienic viewpoint, chiefly because of the danger of irradiation which it presents both for the patient and for medical personnel. The catheterization method has a number of characteristic features which, when compared with ordinary X-ray methods, considerably increase the danger of irradiation. Osborn and Howland have pointed out the danger of irradiation and the need for observing caution in catheterization. Camermann asserts that in cardiac catheterization "the erythema or necrotizing dose may be reached and even exceeded." Jacobson presents the doses which are received by people who are performing cardiac catheterization. To date, no hygienic evaluation has been made of the radiation associated with cardiac catheterization and the personnel carrying out these investigations frequently do not have a clear idea of the degree of danger of the irradiation.

The experimental portion of the work was performed in the Balneological Clinic of the Faculty Hospital at Bratislava. A paraffin phantom was used instead of a patient. The intensity of the primary beam was measured by the general-purpose Simmens dosimeter with a thimble chamber; the secondary irradiation was determined by an intendosimeter (Czechoslovakian patent No 85084). The irradiation was carried out by a four-kenotron X-ray apparatus of the Megamet 125 brand with a Motoskop 14 tilt table and a Met Don X-ray tube. The X-ray tube had a built-in filter of 1 mm Al and additional filters as follows: for a voltage of 60 kv, 1 mm Al; at 70 kv, 1.825 mm Al; at 80 kv, 2.7 mm Al.

We sought an answer to two questions: 1) what doses are received by the patient during cardiac catheterization; 2) what is the distribution of the scattered radiation within the X-ray clinic which threatens the personnel during cardiac catheterization.

There is no doubt that the dose received by patients during any X-ray investigation depends primarily on the intensity of the primary beam. In our X-ray tubes the intensity with the voltage usually used (60-80 kv) and the

anode current usually used (three-four ma) ranges between four and 12 r/minute. The magnitude of the surface dose was measured at the surface of the phantom with a surface-to-tube distance equal to 45 centimeters; the depth dose was measured in the middle of the phantom at a depth, equal to 12 centimeters.

In Table 1 the dose rate is indicated of the surface and the depth dose in the middle of the phantom depending on the magnitude of the field used on the fluoroscopic screen. From the Table it is seen, for example, the dose rate at the surface (voltage 60 kv) is increased by 20 percent when the field is increased from 10 x 10 centimeters to 30 x 30 centimeters, whereas with the voltage of 80 kv the increase is equal to 36.7 percent of the original level. Such a relationship was found also in depth. According to Table 1 the surface and depth doses received by patients during a single examination may be computed.

Table 1

Change in Dose Rate Depending on the Size of the Field (Current 4 ma, Surface-to-tube Distance 45 Centimeters, Filter 1 mm Al)

Size of the field, cm.	10 × 10			20 × 20			30 × 30		
Voltage at tube, kv	60,0	70,0	80,0	60,0	70,0	80,0	60,0	70,0	80,0
Dose rate at surface, r/min	5,0	7,2	9,5	5,5	8,5	11,0	6,0	8,75	13,0
Dose rate at a depth, r/min	0,5	1,0	1,3	0,75	1,5	2,0	1,0	1,7	3,0

The fluoroscopy time proper during cardiac catheterization was equal to 25 minutes (but frequently it was more). The patient receives a surface dose of 150-325 r depending on the magnitude of the voltage.

Table 2 shows that when an X-ray tube is used without a filter the intensity of the dose at the surface is four times greater than when an additional 3-mm. Al filtration is used. The intensity of the dose at a depth in this case was cut in half. However, it should be emphasized that in a conscientious approach to the matter the physician should require that the technicians set up a filter of maximum thickness on the X-ray tube which still permits adequate contrast and brightness in the image. He should do this, first of all, for the sake of the patient but also

for his own safety. Here, we run into the second question -- the distribution of secondary radiation in space.

Table 2

Change In Dose Rate Depending on Use of Various Filters (Current 4 ma, Radiation Field 30 x 30 Centimeters, Surface-to-tube Distance 45 Centimeters, Voltage 70 kv)

Filter, mm Al	0	0.5	1.0	2.0	3.0
Dose Rate at Surface, R/min	16.5	11.5	8.75	5.7	4.0
Dose Rate at a Depth, R/min.	2.1	2.0	1.7	1.2	1.0

Taking into consideration the fact that this method of investigation is not associated with irradiation of the medical worker making the examination with the primary beam, we directed attention to the scattered secondary radiation. With this aim in view an exam ation was made of the spatial distribution of the scattered radiation in the X-ray clinic. The dose rate measured in air and determined in milliroentgens per hour was represented on the diagrams in a single horizontal and two vertical planes in the form of curves with the same intensities (isodoses). This method of recording makes it possible to create quite a clear and accurate concept of the danger for medical workers in certain places near the X-ray apparatus. The isodose curves show the following values in horizontal and vertical beam areas: I -- 1,000 mr/hour; II -- 700 mr/hour; III -- 500 mr/hour; IV -- 400 mr/hour; V -- 300 mr/hour; VI -- 200 mr/hour; VII -- 100 mr/hour; VIII -- 50 mr/hour; IX -- 20 mr/hour; X -- 6.5 mr/hour.

In Fig. 1 the distribution is given of the secondary radiation during cardiac catheterization. The places of maximum danger are seen, which are represented by the scattered radiation, chiefly in the immediate vicinity of the horizontally arranged table on both sides of the screen-support rods if we keep in mind the fact that three or four medical workers stand exactly at these places during the examination, where they may obtain more than twice the maximum permissible daily dose during a single examination, the significance of the application of protection factors becomes clear. Even at places where the worker is located who takes care of the electrocardiograph, that is, at a distance of approximately 200 centimeters from the focus, the dose rate of scattered radiation is equal to 20-50 mr/hour.

From the Figure it may be seen which portions of the body are subjected to the greatest danger: hands, feet, lower portion of the trunk.

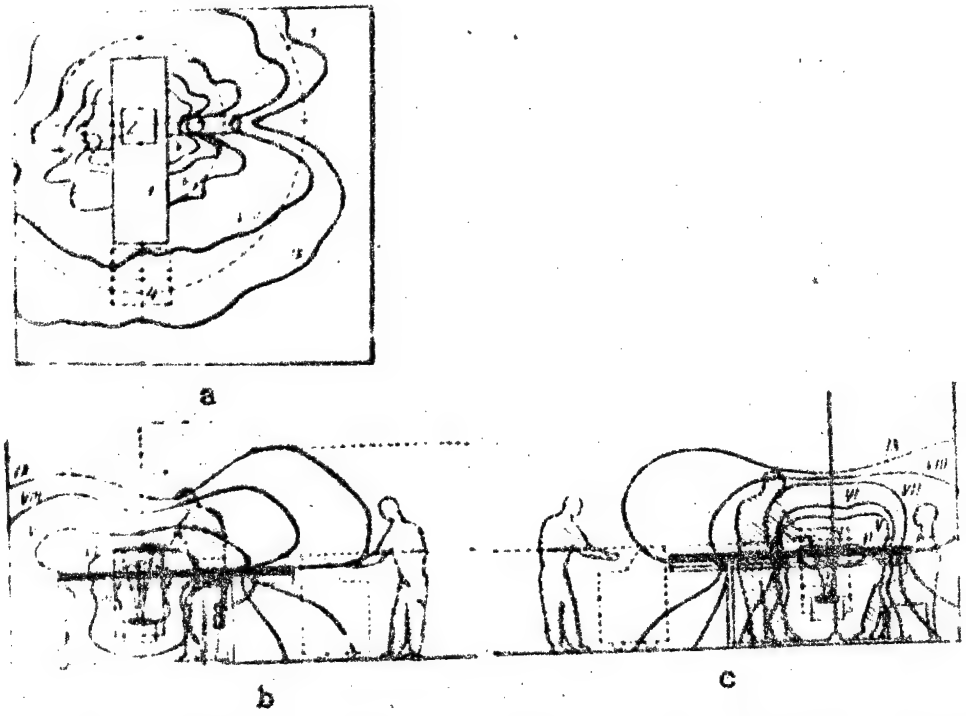

Fig. 1. Distribution of Secondary Radiation in X-ray Clinic with Table in Horizontal Position. Voltage 60 kv, Current 4 ma, Radiation Field 30 x 30 Centimeters; Filter 1 mm Al; Skin-to-tube Distance 45 Centimeters.

a -- horizontal area of beam at a height of 90 centimeters above the floor; b -- vertical area of beam on the left; c -- vertical area of the beam on the right; 1 -- horizontal position of tilt table; 2 -- fluoroscopic screen; 3 -- support rod of X-ray tube; 4 -- electrocardiograph. See the text for the explanation of the arabic numerals.

The distribution of the secondary radiation with a voltage of 70 and 80 kv shows an increase in the dose rate when the voltage at the X-ray tube is increased.

In Fig. 2 the isodoses of scattered radiation are given with a voltage of 80 kv, which are not shown in Fig. 1. A great displacement is seen of all the isodose curves away from the center and the appearance of new curves I, III, and IV which represent levels of 1,000, 500 and 400 mr/hour.

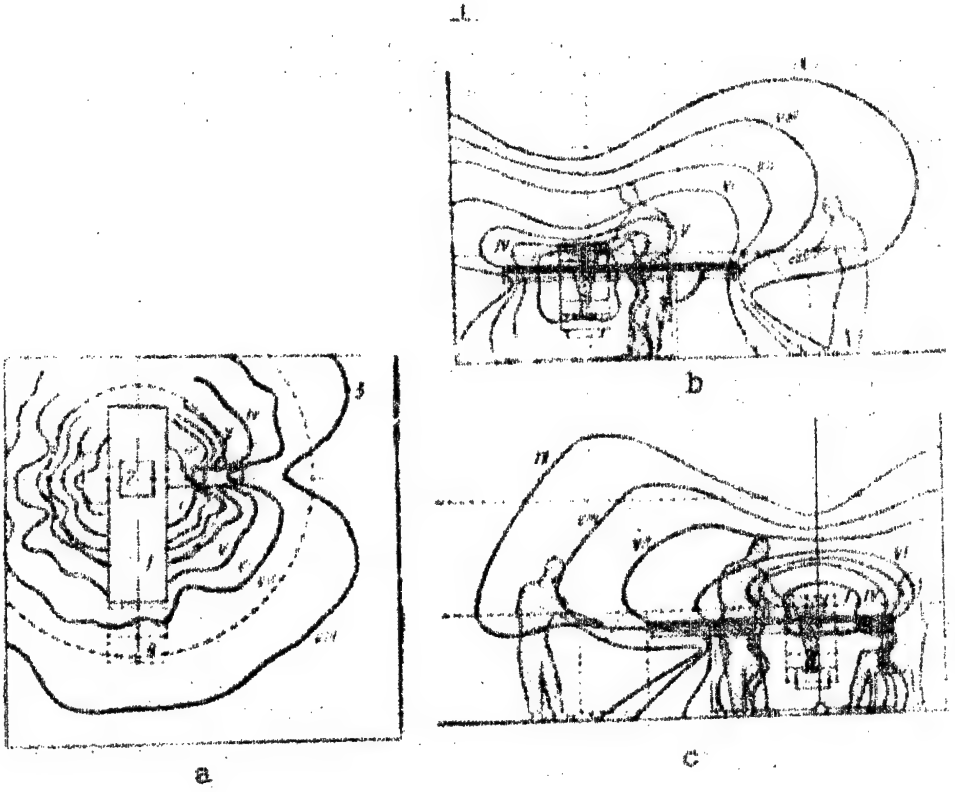

Fig. 2. Distribution of Secondary Radiation in X-ray Clinic with Horizontal Position of Table. Voltage 80 kv, Current 4 ma, Radiation Field 30 x 30 Centimeters; Filter 1 mm Al; Skin-to-tube Distance 45 Centimeters. The key is the same as for Fig. 1.

The effect of the filter thickness is noted on the intensity of secondary radiation without any attached (external) filter. With the use of an additional one-millimeter Al filter at a voltage of 70 kv the intensity of scattered radiation is decreased by almost half in comparison with irradiation without a filter. This is seen, for example, from the fact that whereas the dose rate varies at about 200 mr/hour with the use of a 1-mm Al filter at a distance of 90 centimeters, the dose rate is equal to 300 mr/hour without a filter at the same distance. A suitable filter, therefore, is needed not only for the patient's safety; it plays a part also in reducing the scattered radiation level threatening the medical personnel.

We investigated the relationship between the level of scattered radiation and the magnitude of the field used on the fluoroscopic screen. The results of the investigation showed that the use of a small field is the simplest and, at the same time, the most effective method

of reducing the level of harmful radiation and the danger associated with it. Experienced roentgenologists use this simple method everywhere and always, wherever it is possible.

Conclusions

During cardiac catheterization the patient receives large doses of radiation which can be effectively reduced by increased external filtration and reduction in the fluoroscopic field.

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Methemoglobin-Formation in Radiation Sickness

K. S. Kosyakov

The transformation of hemoglobin into methemoglobin represents an oxidative process in which the iron of the hemin grouping goes from a bivalent into a trivalent state. In pathology we deal with two routes of methemoglobin-formation. The first route is observed when toxins enter the body, which, after coming into contact with the blood, transform hemoglobin into methemoglobin. Among the substances of this kind are nitrites, Berthollet's salt [potassium chlorate], aniline, and red blood salt [hematite]. The second route of methemoglobin-formation is that occurring as the result of the reaction of hemoglobin with endogenous methemoglobin-formers which occur during the process of metabolism.

In 1939 (K. S. Kosyakov) it was shown in in vitro experiments that extracts from various organs form methemoglobin in contact with blood. It was also established that aldehydes, glycerin, and amino acids, that is, products of the intermediate metabolism of carbohydrates, fats and proteins, in contact with hemoglobin solution convert it to methemoglobin. This made it possible to express the idea that methemoglobin-formation is a physiological process which occurs continuously in the blood stream because of the presence of "endogenous methemoglobin formers" in it. However, it is usually impossible to find methemoglobin in the blood, because simultaneously with its formation a continuous reduction of it occurs -- a transformation back to hemoglobin with bivalent iron in the hemin grouping. Actually, as has been shown by the investigations of V. S. Shapot and G. Ye. Vladimirov and others, the products of the intermediate metabolism of carbohydrates, glutathione and ascorbic acid, reduce methemoglobin to hemoglobin. Physiologically, these opposite processes -- the formation of methemoglobin and the reduction of it -- are in balance.

In pathological conditions, this equilibrium is disturbed; methemoglobin-formation begins to predominate over methemoglobin reduction, and methemoglobinemia occurs. This sign can accompany a number of diseases, and can be observed even in persons who are healthy otherwise as a special form of metabolic disorder (a so-called "essential methemoglobinemia"). Soon after these investigations, which determined that methemoglobin formation was a physiological process, the presence of a small quantity of methemoglobin in the blood was established by a number of authors

in healthy animals and people by the use of sensitive spectral methods of analysis.

Z. I. Kozlovskaya and N. N. Savitskiy, using extensive material concerning the clinical aspects of internal diseases, showed that the appearance of methemoglobin in the blood occurs particularly frequently in inflammatory and infectious diseases. In the investigation of 463 blood samples from 386 patients by these authors the presence of methemoglobin was established in 40-47 percent in bronchopneumonia, enterocolitis, hepatitis, acute septic and septicemic conditions. Methemoglobin was found in croupous pneumonia in all ten patients examined. Van Slyke reported the presence of an average of 0.4 percent methemoglobin in the blood of healthy persons. According to the data of Sibuja, there is 0-1.5 percent (an average of 0.62 percent) methemoglobin in the blood of healthy persons. The production of methemoglobin through the effect of ionizing radiation has repeatedly attracted the attention of research workers. In the modern concepts of the effect of radiation a considerable place is given to oxidizing radicals formed during the process of water radiolysis. The idea that the oxidizing radicals in contact with hemoglobin produce a transformation of it to methemoglobin has a sound basis. Actually, as experiments of Guzman-Barron and Jonson have shown, the irradiation of a hemoglobin solution of human blood with X-rays in large doses leads to a general reduction in the absorption maxima in the visible portion of the spectrum and an increase in absorption at a wavelength of 6300 Å, which is evidence of methemoglobin-formation. This methemoglobin -formation is increased with the increase in the radiation dose.

After irradiation with doses of 10,000, 25,000, 50,000, 75,000 and 100,000 r the percentage of absorption is changed at 6300 Å; 5.6; 17.8; 42.2; 54.5; and 82.3, respectively. Dowben and Walker established the fact that irradiation with a dose of 25,000 r 60 percent methemoglobin is formed from lysed human erythrocytes. According to the calculations of Klein, the quantity of oxidizer which could have been formed in the experiments of the authors mentioned above could transform about 50 percent of the hemoglobin into methemoglobin. Therefore, the calculations and experimental results come close to each other. From these calculations it followed that irradiation of rats with a dose of 3,000 r should convert about two percent of the hemoglobin into methemoglobin. In experiments on rats with irradiation with 5,000 r and examination of their blood immediately after irradiation, Klein found a methemoglobin concentration

which did not exceed 0.2 percent, just as in the non-irradiated animals. Apparently, this is evidence to the effect that in the intact organism the circulating products of the intermediate metabolism assure a rapid reduction of methemoglobin formed.

In the examination on the rats, Dowben and Walker, in contrast to Klein, established the fact that methemoglobin occurs after irradiation. In the experiments of these authors, the rats were exposed to a total-body irradiation with X-rays in a dose of 6,000 r. Blood samples were taken before irradiation, at a level of half of the dose, and six-eight hours after irradiation. A consistent increase was established in the methemoglobin concentration to an average of seven percent at the end of the irradiation instead of the original 1.5 percent; after an hour, the methemoglobin concentration returned to the original level, and then increased again to three to four percent.

An indirect idea of methemoglobin formation in dogs after the effect of ionizing irradiation can be obtained from the research of V. V. Marenov, who studied the respiratory function of the blood in acute radiation sickness. According to the data of this author, the gas composition of the blood was essentially unchanged up to the tenth day of irradiation of dogs with a dose of 600 r. On the tenth day a reduction in the hemoglobin was noted to 11.7 percent and in the oxygen capacity, to 15.93 percent. With these relationships the Huffner constant was equal to 1.36, that is, the capacity of oxygen transfer in the irradiated animals is not reduced, which indirectly attests to the absence of any noticeable quantities of methemoglobin.

M. A. Rozhdestvenskaya and T. M. Ostroukhova report the constant occurrence of methemoglobin in the blood of the irradiated animals. According to the data of these authors, the methemoglobin concentration can reach very high figures (of the order of 40-50 percent). The authors consider the cause of methemoglobin formation the occurrence of water radiolysis products in the blood. The contradictory nature of the data in the literature stimulated us to clarify at which period of acute radiation sickness and in what quantities methemoglobin occurs. The elucidation of this matter would make it possible for us to clarify the mechanism of methemoglobin formation in radiation sickness. It was also necessary to determine to what extent this metabolic disorder can interfere with oxygen transport.

We made investigations on male dogs weighing 12-18 kilograms, which were exposed to a single total-body irradiation with X-rays from two RUM-3 apparatuses under the

Following conditions: voltage 180 kv, current 15 ma, filters 0.5 mm Cu; distance, 120 centimeters; dose rate, 3.25 r/min in the first apparatus and 4.15 r/min in the second; total dose, 500 r. As a result of this effect the acute form of radiation sickness occurred in the experimental animals, from which 70 percent of the dogs died during the first two or three weeks of the disease. Before irradiation and during the course of the radiation sickness, blood was taken from the animals from the femoral artery for the purpose of determining methemoglobin concentration. The blood was diluted 200 times with an ammonia solution (0.1 percent) and examined in an SF-2M spectrograph of Soviet production. Spectrograms were made in the range of 5,000-6,000 Å.

For the purpose of determining the percentage concentration of methemoglobin, we made use of the light absorption ratio recommended by Heilmeyer at a wave length of 5,420-5,600 Å. Table 1 served for the purpose of calculation.

Table 1

Computation Table for Determining Methemoglobin Percentage

Methemoglobin percentage	0	5	10	15	20	25	30	35	40	45	50
L_{5420}/L_{5600}	1,63	1,61	1,59	1,57	1,55	1,53	1,51	1,49	1,47	1,45	1,42
	55	60	65	70	75	80	85	90	95	100	
	1,40	1,38	1,36	1,34	1,32	1,30	1,28	1,26	1,24	1,22	

In all, 384 blood samples were examined from 88 dogs. Of these, 149 samples were from animals before irradiation and 235 samples were after irradiation at an interval of from one hour to 49 days. The results of the investigation are presented in Table 2.

Methemoglobin was found only rarely and in small quantities in healthy dogs: in 149 samples, seven percent methemoglobin was found in only one sample, and in six samples the quantity did not exceed five percent. In the other 142 tests there was no methemoglobin. Directly after irradiation, when blood was taken during the first hour, methemoglobin was not found in any of the nine samples. Afterwards, during the first 15 days, when the sickness reached its maximum development and the majority of animals began to die, methemoglobin was found with the same frequency as in the non-irradiated animals: in 126 samples it was found in only five cases, and the quantity of it only once exceeded ten percent. The occurrence of methemoglobin

increased somewhat in frequency during the period between the 20th and 30th day of the sickness; however, even here it was encountered in only 8.35 percent of the samples examined. During the period between the 35th and 49th day after the irradiation, methemoglobin occurred in 42 percent of the samples and not uncommonly amounted to 10-15 percent. The quantity of methemoglobin of over 27 percent was not found even once; even this figure was found in only one sample on the 35th day of the disease.

Table 2

Methemoglobin Concentration in Blood of Dogs With Radiation Sickness (Single Irradiation With Dose of 500 r)

Met-hemo-globin %	Before irradiation	After 1 hour	After 24 hours	After 5 days	After 6-7 days	After 10 days	After 14-15 days	After 21 days	After 25 days	After 28-30 days	After 35 days	After 40-42 days	After 45-49 days	Total
0	142	9	14	28	21	28	30	14	12	18	1	3	1	[illegible]
1—5	6	—	1	1	—	—	—	2	—	—	1	2	—	13
6—10	1	—	—	—	—	1	1	—	—	1	2	1	3	10
11—15	—	—	—	—	—	—	—	1	—	1	2	2	2	7
16—20	—	—	—	—	—	—	—	—	—	—	1	—	—	2
21—30	—	—	—	—	—	1	—	—	—	—	—	—	—	1
Total	149	9	15	29	21	30	31	17	12	19	22	15	15	384

Table 3 gives us an idea of the frequency with which methemoglobin was found during the various periods of the sickness.

Table 3

Frequency With Which Methemoglobin Was Found (In Percentages of Number of Blood Samples) in Dogs During the Course of Radiation Sickness

Time	Before irradiation	After 1-24 hours	After 5-15 days	After 20-30 days	After 35-45 days
% of cases with met-hemo-globin	4.7	4.17	3.2	8.35	42.3

In summarizing the results of the data obtained, we see that the occurrence of methemoglobin in the first hour and during the first day after irradiation is not characteristic. Therefore, it is hard to speak of the significance of water radiolysis products in this process. While

these products actually form a certain quantity of methemoglobin, which is confirmed by in vitro experiments, a reduction of methemoglobin to hemoglobin occurs rapidly in the body by the biochemical systems which accomplish this process during physiological methemoglobin-formation.

The occurrence of methemoglobin in the blood of an animal sick with radiation sickness becomes a regular feature, which is encountered in 42 percent of the cases, only with the expiration of five to six weeks after the irradiation. In these cases, we are dealing with a portion of the animals which survived irradiation with an LD_{70} and are in the stage of recovery, suffering from various associated diseases which occur against the background of a reduction in the immune protection of the body (inflammatory processes in the respiratory and digestive tracts).

By this time the hemorrhagic syndrome and its abundant hemorrhages in various tissues are manifested in an increased form. As is well known, the shed blood is subject to changes in which the hemoglobin is converted into methemoglobin. The color changes of hemorrhages, spectral examinations, and the data of our direct experiments with tissue homogenates (K. S. Kosyakov) are evidence of this. In the presence of increased capillary permeability it is quite natural to expect not only the penetration of blood into the tissues but also the resorption of erythrocyte decomposition products, particularly methemoglobin, into the bloodstream. The methemoglobin concentration in radiation sickness does not reach the levels which impair oxygen transport to any great extent; therefore, radiation methemoglobinemia does not require any special treatment.

Since it appears late as a result of the development of the hemorrhagic syndrome and complications of infectious nature, methemoglobinemia does not have any value for the early diagnosis of radiation sickness and to a certain degree indicates only the severity of the course of its late period.

Conclusions

1. Methemoglobinemia in dogs irradiated with X-ray in a dose of 500 r is not found any more often than in healthy animals during the initial period and during the period of the climax of radiation sickness.

2. During the recovery period, methemoglobinemia is found in 42 percent of the cases in the animals which survive the irradiation and is a consequence of the reaction of hemoglobin with endogenous methemoglobin-formers. The

presence of hemorrhages and complications against the background of reduced immunological protection of the body contributes to this process.

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The Properdine System in Radiation Sickness (Review of the Literature)

I. L. Chertkov

In 1954, the American scientist Pillemer with a group of his co-workers reported on the discovery of a new bactericidal and virus-neutralizing system of the body which was given the name of the properdine system. The main component of the system is a new blood protein -- properdine (from the Latin perdere -- to destroy). The properdine system includes also four components of complement and the magnesium ion.

According to Pillemer's opinion, the properdine system assures the bactericidal nature of the blood and also takes a very important part in natural immunity. Data reported in this work to the effect that after a total-body X-irradiation of rats with a dose of 500 r the properdine concentration dropped as early as after two days from 25-35 to 4-6 units/ /cubic centimeter and after 7 to 13 days the activity of the properdine system of the blood in the irradiated rats was not found at all are of particular interest. The restoration of the properdine content in surviving animals occurred only after several weeks depending on the dose used. Pillemer believes that this reduction in the blood properdine following irradiation explains the well-known fact of the reduction in resistance to infection during radiation injuries. Similar data concerning the dynamics of the changes in properdine titers in rats with acute radiation sickness have been reported by Ross.

Linder and others reported a somewhat different nature of the activity changes in the properdine system in acute radiation sickness in rats. According to their data, the reduction in the properdine level in the blood following irradiation with a dose of 500 r occurs more slowly and to a lesser degree than was reported by Ross. Thus, 24 hours after irradiation, the level of properdine was equal to 93 percent of the original; after three days, 52 percent; after eight days, 34 percent. The recovery in the properdine level in the surviving animals occurs much more rapidly -- 13 days after irradiation the concentration of properdine was approximately 60 percent of the original level. The authors point out that the reduction in the properdine level in the blood is not associated with the hardness of radiation, because irradiation in a betatron or in an X-ray apparatus acted with equal effects on the properdine system.

The nature of the changes in the properdine system in radiation sickness in different species of animals has been studied in particular detail by A. A. Bagdasarov, M. O. Raushenbakh, I. L. Chertkov and others. In the

majority of animal species studied (dogs, mice, guinea pigs, rats) the properdine level decreased usually shortly before death of the animals. Here, the reduction in the properdine level in the blood was associated not so much with the dose of radiation or with the time which elapsed as with the general condition of the animals. The reduction in the activity of the properdine system was an extremely unfavorable prognostic sign and was evidence of the severity of the pathological process and depletion of the compensatory capacities of the body. These relationships were shown with particular distinctness in experiments on dogs. Thus, in animals which died rapidly after irradiation (7th to 11th day) the decrease in the properdine level in the blood occurred as early as on the second to fifth day of radiation sickness; in dogs which died on the 15th-20th day after irradiation, the reduction in activity of the properdine system developed only on the 15th day of radiation sickness. In dogs which survived, no changes in the properdine activity were observed as a rule. However, in the surviving animals in which radiation sickness occurred with particular severity a reduction in the properdine level in the blood was observed. In these cases, normalization of the properdine level occurred quite quickly, coinciding with clinical recovery.

In contrast to the species of animals indicated above it was impossible to note any reduction in the properdine level in the blood in rabbits with acute radiation sickness. Particularly pronounced changes in the activity of the properdine system were noted in investigations on monkeys. In the latter, the reduction in the properdine level was observed immediately after irradiation, whereby it was noted in both surviving monkeys and those which died subsequently. On the day of death a distinct agonal increase in the properdine level was noted in the monkeys which was observed also sometimes in experiments on dogs and rats. In surviving monkeys, the activity of the properdine system remained reduced for a very long time; normalization of the properdine level lagged considerably behind clinical recovery.

Therefore, in these works definite species differences were demonstrated in the reaction of the properdine system to irradiation, which to a certain degree reflected the condition of the species radioresistance. Thus, in the rabbits most resistant to irradiation the properdine system was practically unaffected, whereas in monkeys highly sensitive to irradiation the properdine system was very labile. In the more radioresistant rats, the properdine system was less affected than in the less resistant mice and the guinea pigs.

The data presented show that the changes in the activity of the properdine system are capable of reflecting the condition of radioresistance of the body, and the properdine system itself apparently participates in creating the general body resistance to the effect of irradiation as a pathological factor. However, the role of the properdine system in protecting the body against radiation bacteriemia and autoinfection has evidently been somewhat overestimated by Pillemer. The properdine system itself cannot play the decisive part either in the phenomena of natural immunity or in the creation of the bactericidal nature of the blood. As is well known (Wardlaw and others), only certain gram-negative microbes are sensitive to the bactericidal effect of the properdine system; the sensitivity to properdine is a property of the microbial strain rather than of the microbe species. More than half of the strains of a properdine-sensitive flora proved to be properdine-resistant. In connection with this, autoinfection in radiation sickness can develop also against the background of a normal properdine level because of properdine-resistant strains. It is also well known (N. N. Klemparskaya and others) that radiation bacteriemia develops as early as the first few days after irradiation, whereas the reduction in the properdine level occurs later, as a rule. In rabbits, no reduction in the properdine level in the blood occurs at all in severe radiation sickness, whereas bacteriemia and autoinfection are quite pronounced in them. It should be noted that in chronic radiation sickness the activity of the properdine system does not suffer (L. T. Tutochkina; Hinz).

The mechanism of reduction in the properdine level in the blood in acute radiation sickness has not as yet been determined. It is well known that this mechanism is indirect, because irradiation of the serum in the test-tube using a betatron (500 r did not reduce the properdine activity (Linder and others). The effect of irradiation on properdine synthesis is not very probable either, because reduction in properdine activity is observed with the use of low doses of radiation (500-700 r) which do not exert any effect on the synthesis of other proteins in the body. On the other hand, although the half-life period of properdine is definitely shorter than that of globulin (Keller and others) it is hard to imagine that the disappearance of properdine from the blood in certain cases as early as 24 hours after irradiation can be explained by the stoppage of synthesis of it in the body. Probably, it is a question of binding of the properdine by some substances liberated from the tissues as a result of radiation trauma. Apparently, these substances are of a

polysaccharide nature, because the tissue polysaccharides are capable of binding properdine (Landy, Shear).

Taking into consideration the fact that an increase in the mucopolysaccharides have been noted in the blood in radiation sickness (L. T. Tutochkina), it may be considered that the latter account for the binding of properdine. In special investigation (I. L. Chertkov, Z. I. Sheremet) it has been shown that in dogs, mice and guinea pigs the reduction in the properdine level is regularly associated with an increase in the mucopolysacchride concentration in the blood at the same time. These facts confirm the decisive role of mucopolysacchrides in the mechanism of decrease in properdine in acute radiation sickness; however the problem needs further study.

Attempts at affecting the properdine system with the aim of prophylaxis and therapy of radiation sickness are of particular interest. The works of this kind can be divided into two groups: treatment of radiation sickness by purified properdine and the use of high molecular-weight polysacchrides capable of increasing the properdine level in the body.

Even before the discovery of the properdine system, it was reported (Strond and others) that a protective substance could be isolated from Cohn's fraction III of the serum; the intravenous injection of ten milligrams of this substance into mice several minutes before irradiation reduce the mortality rate of the animals, which had received a total-body irradiation with a dose of 800 r, to a mortality rate level of that produced by 500 r. In the light of the modern data it may be considered that the effect is explained by the action of properdine, which is present in Cohn's fraction III.

According to the data of Pillemer and Ross, the intravenous injection of properdine obtained from cows (in a dose of 50 units) into mice irradiated with a dose of 820 r 2, 24, and 48 hours after the irradiation saved 14 out of 22 mice, whereas there was 100 percent mortality in the controls. After the intravenous injection of 250 units of properdine into rats on the second, fourth and seventh day after irradiation with a dose of 660 r ($LD_{90/30}$) definite protection was obtained (Pillemer and others). It should be pointed out that the properdine effect is intimately associated with the time which has elapsed after irradiation. Thus, according to the data of Ross, the intravenous injection of 50 units of properdine into mice irradiated with a dose of 600 r on the second, third and fourth or tenth day after irradiation did not save the animals, whereas the same dose of properdine injected on the seventh day after irradiation saved 7 out of 16 mice (in the controls 7 out of 64 mice survived).

The increase in the survival rate of mice from radiation sickness under the influence of properdine has been noted also by I. A. Pelishenko and others. In rats the injection of properdine produced an increase in the bactericidal activity of the blood in a part of the cases, with an increase in the leukocyte and pletelet count, normalization of the processes of blood coagulation, and bone-marrow hematopoiesis. It remains unclear whether the protective effect of properdine in acute radiation sickness is associated with its specific activity, because, on the one hand, properdine is effective also after boiling, which completely destroys its specific activity (Ross); on the other hand, a protective effect of properdine has been noted also after intraperitoneal injection (I. A. Pelishenko and others), although, as is well known, the entire properdine molecule does not pass through the peritoneum.

All this is evidence of the necessity for further study of the problem of utilization of properdine for the therapy of acute radiation sickness. The development of this problem is impeded by the need for utilizing very large quantities of properdine. It is sufficient to state that the dose of properdine used in the works of Pillemer and Ross exceeded the total concentration of properdine in mice by two to five times. If the smaller doses proved to be ineffective (and this problem has not been studied as yet), 10,000-20,000 units of the purified properdine will have to be injected into man for the purpose of obtaining a protective effect, which is, in practice, very complicated. Therefore, the use of preparations which considerably increase the properdine level after injection into the body is more promising.

At the present time, the capacity of increasing the properdine level in the blood after parenteral injection has been shown by an insoluble polysacchride obtained from yeast cells, zymosan (Pillemer, Ross), levan, dextran, mucin, etc. (Pillemer and others), in various polysacchrides and lipopolysacchrides obtained from microbes (chiefly from gram-negative flora), plants and animals (Büsing; Eichenberger and others; Landy; Landy, Pillemer; Martin; Martin, Voss; Pillemer and others), in certain simple polypeptides (Auerswald), etc. A few of these preparations have been studied as agents for the prophylaxis and therapy of radiation sickness.

Data on zymosan have been given in greatest detail in the literature. In 1955 Pillemer reported that the injection of zymosan 24 hours before irradiation, or 24 hours after it saved up to 70 percent of the mice irradiated

with a dose of $LD_{100/30}$. This problem has been studied more fully by Ross. The results of his investigations are presented in Table 1.

Table 1

Mortality Rate of Mice Irradiated With a Dose of 600 r Treated by the Injection of Zymosan

Dose of zymosan (in mg per mouse)	Time of injecting zymosan after irradiation, hours	No of surviving mice in the total No of 24
Control	—	7
0,1	24	15
0,1	48	1
2,5	24	1[illegible]
2,5	48	1[illegible]

As is seen from Table 1, zymosan exerted a definite protective effect, even when injected after irradiation.

In the work published in 1958, Ross points out that zymosan is maintained for a long time in the body and is activated on subsequent irradiation of the animals. Thus, irradiation of mice (600 r) which were given zymosan three months previously produces an increase in the properdine level of one and a half to two times after 24 hours, whereas in control mice the properdine level in the blood decreased by 60 percent 24 hours after irradiation according to Ross' data. It should be noted that A. A. Bagdasarov and co-workers observed an increase in the activity of the properdine system 24 hours after irradiation in mice which had never been given zymosan.

In a number of works of Soviet authors (V. M. Alekseyva and others; G. A. Gankevich and others; G. A. Gankevich) a beneficial effect of zymosan was noted on the course of radiation sickness. After the injection of 125 milligrams/kilogram of zymosan three days before irradiation an increase was noted in the survival rate of the mice. The plasma of dogs obtained after the injection of zymosan exerted a more pronounced therapeutic effect in acute radiation sickness than the plasma of intact dogs. The injection of zymosan increased the bactericidal power of the blood, stimulated leukopoiesis, brought about leukocytosis and phagocytosis.

The evaluation of the efficacy of zymosan in acute radiation sickness, however, remains contradictory. A

detailed check of Pillemer's and Ross' data was made by Haley and others. In experiments on 600 mice they established the fact that the injection of zymosan in doses of from 5 to 125 milligrams per kilogram 14, 7, and 3 days or one hour before irradiation (550 r) as well as at the same intervals after it did not exert any beneficial effect on the course of radiation sickness, but large doses of zymosan (125 milligrams per kilogram) even accelerate the death of the animals. The efficacy of zymosan in acute radiation sickness was not confirmed by Smith (quoted by Haley and Linder and others) either. At the same time, there is a considerable number of works which note an increase in the radioresistance after the injection of various microbial preparations. Thus, N. N. Klemparskaya and others point out that the injection of typhoid vaccine into mice 14, 7, and 3 days before irradiation (400-500 r) decreases the mortality rate of the animals by eight to ten times. A similar effect was produced in rabbits by the injection of paratyphoid A vaccine, the colon bacillus, the bacillus of Flexner dysentery, and in guinea pigs, by the BCG vaccine.

It is not possible, however, to ascribe a protective effect of all the preparations mentioned above simply to the activation of the properdine system, mainly because the increase in resistance to infections is observed also after the injection of lipopolysacchrides, which do not affect the properdine system, and after the injection of BCG, and pertussis vaccine. On the other hand, increase in resistance was observed also to the gram-positive flora (pyogenic staphylococcus, the tubercle bacillus) which is insensitive to the effect of the properdine system (Dubos and others; Nanni). Apparently, the effect of these preparations to a considerable degree is associated with their capacity of stimulating the reticulo-endothelial system, the production of antibodies, increasing the bone marrow function, producing a pyrogenic reaction, and increasing the resistance to trauma and blood loss (Benacerraf and others; Boeme and others; Zweifach and others).

As is seen from what has been stated the significance of the properdine system in acute radiation sickness is far from being proved. Further investigations should establish the nature of the changes in the properdine system in men in acute radiation sickness, demonstrate the mechanism of the reduction in properdine concentration in the blood, the role of the latter in the general resistance of the body, the possibilities of utilizing properdine and of high molecular-weight polysaccharides for purposes of prophylaxis and therapy of radiation sickness. A solution of these problems may introduce clarity into many complicated problems of the

pathogenesis and therapy of acute radiation injuries.

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BRIEF COMMUNICATIONS

The Effect of X-rays on the Concentration of Lactic Acid, Adenosinetriphosphoric Acid, Creatine Phosphate and Inorganic Phosphorus in the Brains of Rats

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The biochemical composition of brain tissue was investigated in 137 white rats which were divided into the following groups: control groups and those irradiated with X-rays in doses of 500-900 r (voltage 170 kv, current 23 ma, field 10 x 15 centimeters, filter 0.5 mm Cu, skin-to-tube distance 30 centimeters). The entire animals were frozen in liquid oxygen, and the concentration of lactic acid, phosphorus, ATP, creatine phosphate and inorganic phosphorus were determined in the brain tissue. As is seen from the Table, after irradiation with a dose of 500-700 r, a tendency occurs toward a reduction in the level of ATP in the brain tissue. In the second week after irradiation with a dose of 900 r and against a background of severe radiation sickness a distinct, statistically reliable reduction in the level of lactic acid and an increase in the ATP are noted in the brain tissue.

The Effect of Irradiation With X-rays on the Chemical Composition of Rat Brain Tissue

Time	Dose, R	Time animals were killed	Elements of variation in a single series	Lactic acid, mg %	Phosphorus, mg %		
					ATP	Creatine phosphate	Inorganic
1 st	Control		N	46,0	29,0	24,0	24,0
			V	24—83	10,3—27,4	5,1—13,8	12,1—41
			M	41,3	18,59	7,18	24,82
			± s	11,8	4,04	0,6	8,43
			± m	1,75	0,76	0,12	1,75
2 nd	[illegible]	After 2 days	N	20,0	12,0	—	—
			V	17,2—87,0	8,8—12,9		
			M	44,3	10,78		
			± s	5,6	3,96		
			± m	1,28	1,19		
3 rd	[illegible]	After 7–9 days	N	10,0	10,0		
			V	31,3—55,3	5,9—16,49	—	—
			M	42,1	11,7		
			± s	6,52	7,95		
			± m	2,17	2,65		
4 th	700	After 2 days	N	8,0	8,0		
			V	21,2—82,7	7,06—20,66	—	—
			M	45,7	11,21		
			± s	6,31	4,70		
			± m	2,38	1,77		
5 th	700	After 10 days	N	12,0	12,0	9,0	9,0
			V	20,5—107,8	5,0—12,41	6,3—10,6	11,0—25,0
			M	55,4	9,02	7,72	18,2
			± s	24,3	6,22	0,42	4,24
			± m	7,32	1,87	0,14	1,3
6 th	900	After 1–5 days	N	33,0	31,0	33,0	33,0
			V	30,0—57,2	9,2—14,1	5,14—12,6	10,2—63,3
			M	40,4	10,92	7,15	30,5
			± s	4,14	4,79	0,5	4,8
			± m	0,73	0,87	0,19	0,85
7 th	900	After 7–14 days	N	8,0	8,0	8,0	8,0
			V	18,0—41,4	15,4—33,0	5,88—10,16	19,3—41,3
			M	29,4	24,86	7,23	35,8
			± s	6,13	6,64	1,82	9,4
			± m	2,31	2,5	0,68	3,56

The Effect of Insulin in Irradiated Animals

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The investigation of carbohydrate metabolism in irradiated animals after the injection of insulin is of practical interest, because the suggestion has been made that insulin be used as a measure for treating radiation sickness (N. N. Blokhin and co-authors).

The aim of the present work was a clarification of the effect of insulin dynamically depending on the dose of radiation.

The experiments were performed on rabbits weighing 2 to 2.5 kilograms. The animals were kept on a constant diet, and were used in the experiment 18-20 hours after their last feeding. In the blood taken from the external auricular vein the concentration of sugar was determined (total of reducing agents) according to the Hagedorn-Jensen method on a fasting stomach, before the injection of insulin and 15, 45, 90 and 150 minutes after the injection.

After preliminary study of the variations in the blood sugar level before and after giving insulin to animals they were subjected to a single total-body irradiation with X-rays. The irradiation was carried out on an RUM-3 apparatus under the following conditions: voltage 180 kv, current 4 ma, filters 0.45 mm Cu and 2 mm Al; skin-to-tube distance of 30 centimeters; total dose of irradiation 100, 200, 400, 600 r. After the irradiation, blood was examined after one hour, twenty-four hours, on the fourth-fifth day, and subsequently every three or four days for three months if the animals did not die. Against the background of irradiation the animals were injected subcutaneously with insulin in a dose of one unit per kilogram, and the concentration of blood sugar was determined.

The behavior of the rabbits irradiated with doses of 100-200 r was no different from the normal. The condition of the animals after the effect of 400-600 r was the usual one only during the first two days; after three-four days a certain sluggishness was noted, and a limitation of movement. The life span of the irradiated rabbits which had been given insulin was shortened by comparison with the control animals. There were no other changes observed in the condition of the animals.

Irradiation of the rabbits with a dose of 100 r did not produce any changes in the sugar concentration in the blood; only in one experiment one hour after irradiation was a certain increase in the sugar level noted from 103 to 123 milligrams percent. When the radiation dose was doubled, that is, 200 r, contradictory results were obtained: in some rabbits there were no changes observed in the sugar level; in others, on the fourth day after irradiation a pronounced hyperglycemia was observed, which was maintained for two or three weeks.

Total-body irradiation of the rabbits with a dose of 400 and 600 r produced an increase in the sugar level in the blood beginning with the first few days after irradiation. After giving insulin to the non-irradiated animals the reduction in the blood sugar level did not exceed 25-40 percent, as a rule. In an animal irradiated with a dose of 100 r the nature of the glycemic curve after the injection of insulin remained similar to that observed in the non-irradiated animal. Insulin injected a long period of time after irradiation (eight to ten months) caused a more pronounced hyperglycemic reaction than in the control animals: a reduction in the blood sugar concentration equal to 60-75 percent of the original level.

In rabbits in which no hyperglycemia was observed after irradiation with a dose of 200 r a reduction in the sugar concentration in the blood was noted by 40-50 percent after the injection of 0.5 units per kilogram of insulin, whereas before irradiation this dose of insulin caused a drop in the sugar level by only 25-35 percent. In these animals, two months after the effect of X-rays the sensitivity to insulin increased considerably. An index of the latter was a reduction in the sugar concentration in the blood by 60 percent from the same dose of insulin. In rabbits with a hyperglycemic effect after irradiation with a dose of 200 r the injection of insulin according to a calculation of one unit per kilogram caused a drop in the sugar level in the blood by 40-50 percent. The insulin effect was more pronounced three months after irradiation. The same dose of insulin reduced the sugar level in the blood by 60-70 percent.

The injection of insulin into rabbits during the first few days after irradiation with a dose of 400 r produced a decrease in the blood sugar concentration such as was observed in the non-irradiated animals. Afterwards, the sensitivity to insulin increased. Thus, for example, two weeks after irradiation the injection of insulin produced a decrease in the blood sugar concentration by 35-50 percent; after six weeks, by 50 percent or more; after eight-ten weeks, by 70 percent.

In rabbits irradiated with a dose of 600 r the blood sugar concentration after injection of insulin on the fifth day decreased by 60 percent, and on the fourteenth day, by 70 percent.

Insulin hypoglycemia in irradiated animals was not always accompanied by a convulsive reaction even when the blood sugar level dropped to 24 milligrams percent.

The results which we obtained confirmed the data of a number of investigators to the effect that the total-body X-irradiation of rabbits is associated with glycemic changes. In some animals a hyperglycemia is noted the degree of which is related to the dose of radiation; in others, with a small dose or radiation no noticeable changes are found in the sugar concentration. The injection of insulin into irradiated rabbits is associated with a more pronounced hypoglycemic effect by comparison with normal animals. The degree of the hypoglycemic effect is directly related to the time which has elapsed after irradiation.

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The Role of Infection and Change in the Immunological Reactivity in the Development of the Hemorrhagic Syndrome of the Irradiated Organism

N. N. Klemparskaya and V. F. Sosova

In radiation sickness a number of phenomena are observed which are characteristic of an allergic reaction of the body -- leukopenia, fever or hypothermia, reduction in the complement titer, diarrhea and hemorrhage. Therefore, it may be supposed that the hemorrhagic phenomena under conditions of irradiation are associated with the development of the state of allergy.

In our opinion, two factors play an important part in the pathogenesis of the hemorrhagic syndrome in radiation sickness: 1) sensitization of the body by tissue decomposition products which occur as a result of the effect of radiation; 2) the so-called "reacting factor" in the form of a local accumulation of increased concentrations of tissue decomposition as a result of tissue destruction by infectious agents or the death and destruction of cells in various organs as a result of an impaired metabolism in progressive radiation sickness.

For the purpose of confirming the part of sensitization and allergy (by tissue products) in the pathogenesis of hemorrhagic changes in radiation injuries it is necessary to obtain experimentally a local hemorrhagic reaction to the injection of homologous tissue products and microorganisms in irradiated animals and non-irradiated animals which are, however, sensitized by these products. An analysis of our own experimental material and the data of other authors permits us to draw a conclusion concerning the correctness of the principle which we proposed regarding the significance of the two factors mentioned. First of all, it was established that in the irradiated organism a local hemorrhagic reaction develops to the injection of homologous tissue products. The hemorrhagic-necrotic changes were obtained by N. N. Klemparskaya in conjunction with N. A. Krayevskiy and V. V. Shikhodyrov. This reaction was observed after the injection of suspensions of intestinal tissue into the skin of the upper lip of mice on the third day after irradiation with a dose of 600 r. The formation of hemorrhages was absent after the injection of this tissue into all the animals and during the first day after irradiation.

For the purpose of proving the role of the process of tissue sensitization it was possible to obtain hemorrhagic phenomena in non-irradiated animals which were given sensitizing and reacting injections of homologous tissue products.

Usually, only a slight hyperemia and a mild diffuse infiltrate could be seen in response to a slight intracutaneous injection of extracts or homologous tissue suspensions into rabbits. After the repeated injections of this material the nature of the reaction changed considerably in the direction of a more intense inflammation with the appearance of hemorrhages.

The role of infection in the development of hemorrhages was studied on rabbits. The infection of irradiated (800-1100 r) rabbits by the injection of microbes intracutaneously (M. M. Izrael'son and others; V. F. Sosova) or into the lungs (A. Ye. Ivanov and V. F. Sosova) produces inflammation characterized by hemorrhagic changes. Because the microorganisms tested in these experiments (colon bacillus, staphylococcus, pneumococcus) do not belong to the group of microbes capable of producing a hemorrhagic reaction, there is no basis for associating the occurrence of hemorrhages at the sites of the microbe injections in the irradiated animals with the properties of the infectious agents.

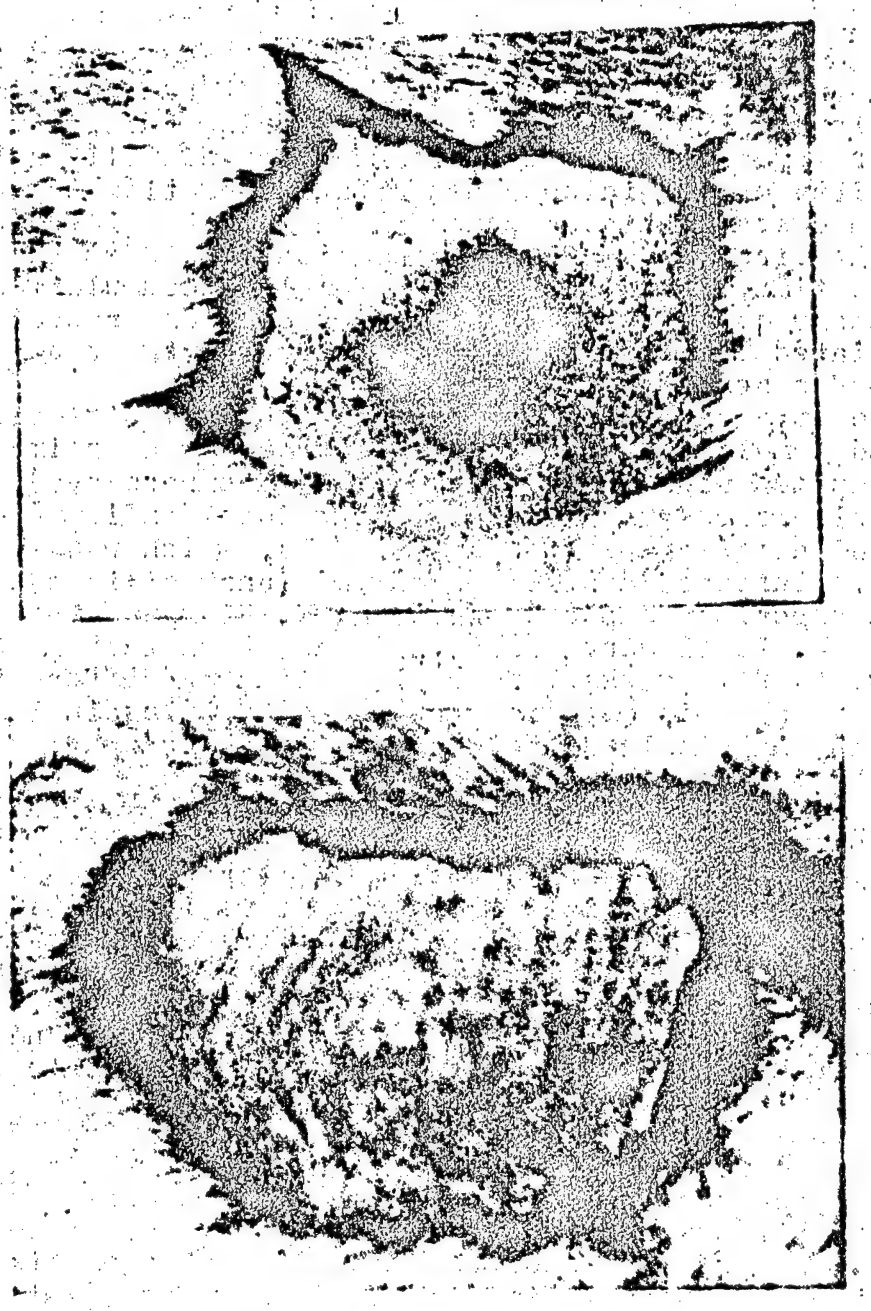
Certain authors (A. Yugenburg and others; P. N. Kiselev; P. D. Gorizontov) have expressed the idea that the irradiated organism acquires an increased sensitivity to its own microflora. However, the infection of autogenous cultures of colon bacillus (V. F. Sosova) did not produce any more pronounced reaction by comparison with that observed after the use of laboratory cultures, and the intracutaneous injection of the same killed microbes (V. F. Sosova) or filtrates of bouillon cultures (Becker, V. F. Sosova), on the other hand, produced even a smaller reaction in the irradiated animals than in the non-irradiated animals. Therefore, on the basis of these data it is justifiable to conclude that there is no specific sensitization to the microbial agent under radiation conditions and, in connection with this, that sensitization has no part in the development of the hemorrhagic reaction to the injection of microbes. Specifically, the products of tissue destruction by microbes appear to be the reacting factor in the allergic reaction, the result of which is the occurrence of hemorrhages.

The occurrence of hemorrhagic changes after experimental infection is observed as early as the third day after irradiation in lethal and sublethal doses. During this period of the acute course of radiation injury there are no manifestations of the hemorrhagic syndrome, although there is a basis for it, such as increased capillary fragility, reduction in the platelet count and an increase in the blood coagulation time. The injection of microbes which destroy the tissues are prepared products of tissue decomposition essentially shortened to the time of the natural development

of hemorrhagic changes in the area of the body of the irradiated organism investigated. The death of the animals without signs of hemorrhagic syndrome during the early periods after irradiation in very large doses as well as the impossibility of obtaining a hemorrhagic reaction in response to certain doses of tissue products or microbes immediately (during the first day) after irradiation emphasize the significance of time, which is required for the accumulation of the necessary quantity of tissue decomposition products in the irradiated organism for the formation of the reaction in the form of a sensitization.

The intracutaneous injection of the colon bacillus into sensitized homologous animal tissue leads, as experiments have shown on guinea pigs and rabbits (N. N. Klemparskaya), to a considerably more severe course of the local inflammatory process and death of part of the animals, which was never observed in infected control organisms (unsensitized).

In experiments performed by N. N. Klemparskaya, R. V. Petrov and L. I. Il'yina (1956), it was shown that a preliminary (two to five weeks before) injection of different fractions of the microscopic structures of intestinal mucosal cells of the rabbit leads to a change in their usual reaction to the intracutaneous injection with 1,000,000,000 microbial bodies of a 24-hour agar culture of the colon bacillus. In place of a small hyperemic infiltrate (in the control rabbits) hemorrhages, necroses and extensive infiltrates (see Figure) developed in the sensitized animals. It is interesting that of nine rabbits which were given injections of cytoplasm and mitochondria of intestinal cells of a healthy rabbit the hemorrhagic reaction was not noted after infection of them with B. coli, although the area of the infiltrates and the intensity of hyperemia were greater than in the control animals. A much more severe course of the local inflammation was noted in animals sensitized by similar cell fractions which were, however, obtained from irradiated rabbits. In six out of nine rabbits the presence of an intense hemorrhagic-necrotic reaction was noted at the site of the intracutaneous infection. In order to show that the formation of tissue decomposition products is important in this case (which was formed under the influence of microbial activity), part of these rabbits were injected intracutaneously with 0.1 cubic centimeter of turpentine on the opposite side of the body, which also produced the occurrence of hemorrhages. Therefore, sensitization by homologous tissue preparations changes the nature of the inflammatory reaction to the injection of microbes in the direction of an aggravation of it and the occurrence of hemorrhages.



Exterral View of Inflamed Foci Upon Injection of One Billion Microbe Cells of a 24-Hour Agar Culture of a Colon Bacillus.
Top -- rabbit previously sensitized by a fraction of cell cytoplasm of an irradiated rabbit; necrosis and hemorrhage are seen at site of injection.
Bottom -- a non-sensitized rabbit.

Therefore, in different variants of the experiments a relationship was established between the occurrence of

the hemorrhagic inflammatory process and the presence of the state of sensitization in the body to homologous tissue products. This sensitization can be created after the injection of suspensions of homologous tissue or fractions of it or as a result of a tissue decomposition produced by the effect of ionizing radiation. The reacting factor which produces the formation of the hemorrhagic reaction is a local production of tissue decomposition products as a result of a disturbance in metabolism and in cell activity after irradiation or from the effect of living bacteria which multiply in them. The direct local injection of homologous tissue products into irradiated organisms or organisms sensitized with homologous tissues shows a similar effect.

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CRITIQUE

G. Mathé, H. Jammet, B. Pendic, L. Schwarzenberg, J. F. Duplan, B. Maupin, R. Latarjet, M. J. Larrieu, D. Kalic, Z. Djukic. Transfusions et greffes de moelle osseuse homologue ches des humains irradies a haute dose accidentellement [Transfusions and Grafts of Homologous Bone Marrow in People Irradiated Accidentally].

S. Salmon. Techniques d'appreciation quantitative des deux populations d'hematies chez quatre sujets irradiés a haute dose et traités per transfusions de moelle osseuse [Techniques of Quantitative Determination of Two Blood-Line Populations in Four Subjects Irradiated With High Doses and Treated With Bone Marrow Transfusions].

In the previous issue of the journal "Meditsinskaya radiologiya," the first of three articles was published devoted to a description of the acute radiation syndrome in six Yugoslavian scientists who suffered from an accident occurring in a nuclear reactor. Below, material is being presented which is published in the other two articles. In them additional data are presented concerning the specific therapy of the radiation injury, and the possibilities of bone-marrow grafting are discussed in detail.

In Table 1 information is presented concerning the age of the patients, the doses of radiation which they obtained, and the specific therapy used.

Table 1

Data Concerning the Age, Sex, Doses of Radiation Received and Transfusion of Bone Marrow from Adult Donors

Initials of patients	Sex	Age	Dose, rem (determined by physical method)	Dose, rem (calculated with consideration of irradiation conditions and clinical picture)	Date of bone marrow transfusion	Volume of bone marrow fluid, cc	No of nucleated cells injected
V.	M	21	840	1000—1200	11 XI	211	$8,5 \cdot 10^9$
M	M	25	876	700—1000	11 XI	183	$11,0 \cdot 10^9$
G.	M	27	920	700—1000	17 XI	270	$12,0 \cdot 10^9$
D.	F	25	1024	700—1000	17 XI	300	$8,5 \cdot 10^9$
H	M	25	696	600—800	20 XI	300	$14,0 \cdot 10^9$
B	M	35	408	300—500	—	—	

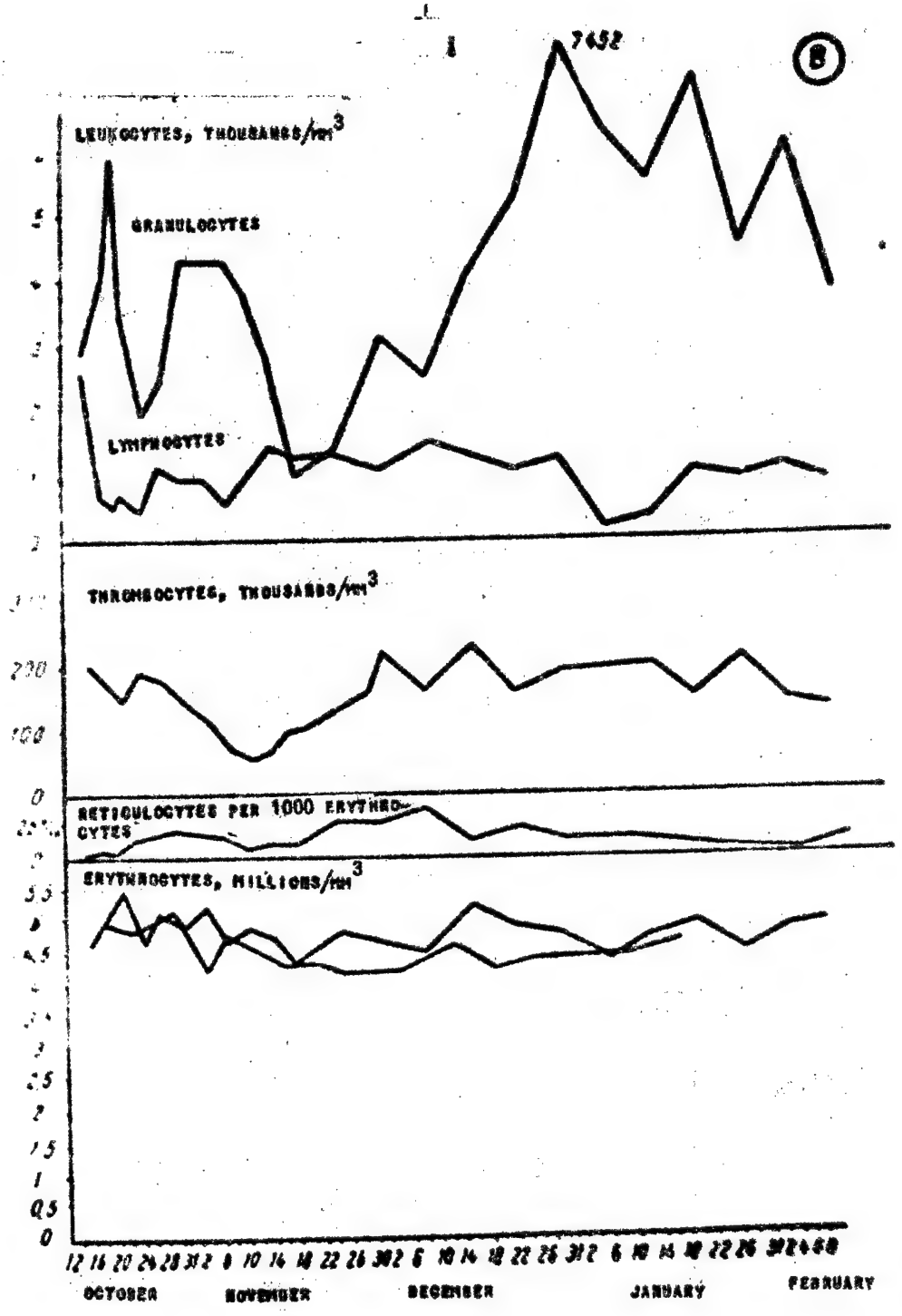
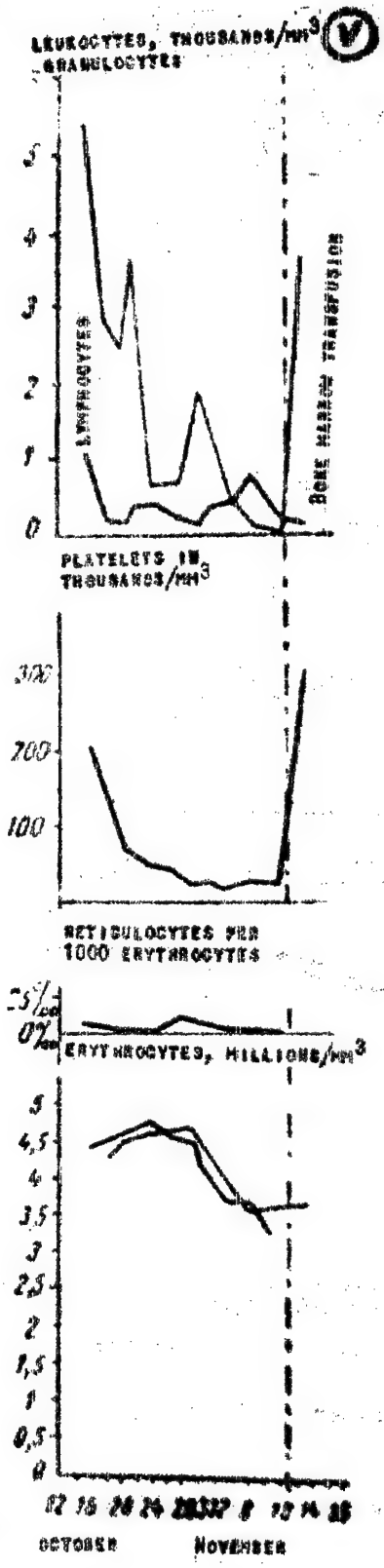


Fig. 1. Changes in the Number of Peripheral Blood Cells in Patient B.

In Figs. 1-6 hemograms are presented from each of



the afflicted persons. As is well known, patient V died, despite the infusion of the bone marrow emulsion, on the 32nd day after the injury. The infusions exerted a definitely favorable effect in the other patients, which was expressed in an improvement in the general feeling of well-being and a change in the blood picture in the direction of a normalization of it: a rapid increase in the reticulocyte and platelet count. At the same time, a decrease in the body temperature to normal, a return of the appetite and an increase in the body weight were noted.

The bone marrow was taken from healthy donors which were selected according to the phenotype of the blood group for each recipient. The investigations of the blood group types were accomplished by the method of differential agglutination according to Ashby in the Würmser modification.

Fig. 2. Changes in the Number of Peripheral Blood Cells in Patient V.

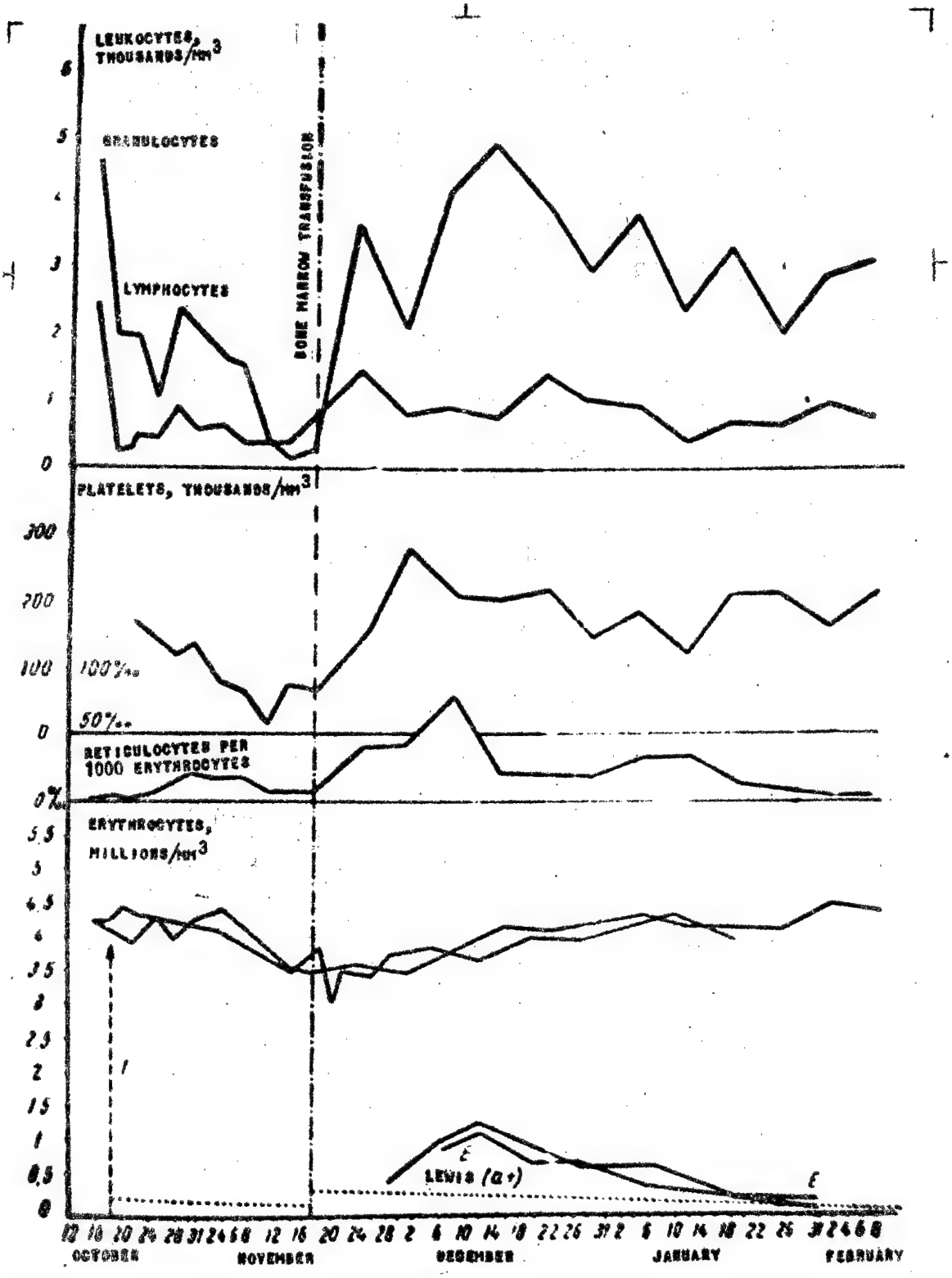


Fig. 3. Changes in the Number of Peripheral Blood Cells in Patient G.

Curves E and Lewis (a+) showed changes in the erythrocyte populations formed by bone marrow injected into the blood and by the recipient's bone marrow; E -- a curve of host erythrocyte populations; Lewis (a+) -- curve of erythrocyte populations determined by the Lewis antigen (characteristic of erythrocytes produced by grafted bone marrow). The arrow indicates the time of blood transfusion.

In Table 2 data are presented concerning the blood group phenotypes of recipients and the donors selected for them. In Figs. 3-6 the curves are presented which illustrate the blood groups to which the erythrocytes belong in the peripheral blood of patients at different periods after the infusion of bone marrow into the donor or recipient. From an analysis of these curves it may be concluded that after the infusion of bone marrow the erythrocyte populations of donor origin first increase and then, over the course of a month, decrease. During the period of increase, these curves are parallel to the concentration of erythrocytes in the blood of the recipients. In Table 3 the sera are indicated which were used for differentiating the erythrocytes.

Table 2

Comparison of Blood Phenotypes of Four Pairs: donor-recipient

1. PATIENT M O	MN	CC Дee	кк F y	(a+)	Jк (a+)	Le (a−в−)
DONOR R O	M	Cc Дee	кк F y	(a+)	Jк (a−)	Le (a−в+)
2. PATIENT G (WOMAN) O	MN	cc ДЕ	кк F y	(a+)	Jк (a+)	Le (a−в−)
DONOR D (WOMAN) O	MN	Cc Дee	кк F y	(a−)	Jк (a−)	Le (a−в+)
3. PATIENT G O	MN	CC Д Ee	кк F y	(a+)	Jк (a+)	Le (a−в+)
DONOR B O	M	CC Д ee	кк F y	(a+)	Jк (a+)	Le (a+в−)
4. PATIENT N A₂	MN	cc Д E	кк F y	(a+)	Jк (a+)	Le (a−в−)
DONOR S A₂	M	Cc Д ee	кк F y	(a+)	Jк (a+)	Le (a+в−)

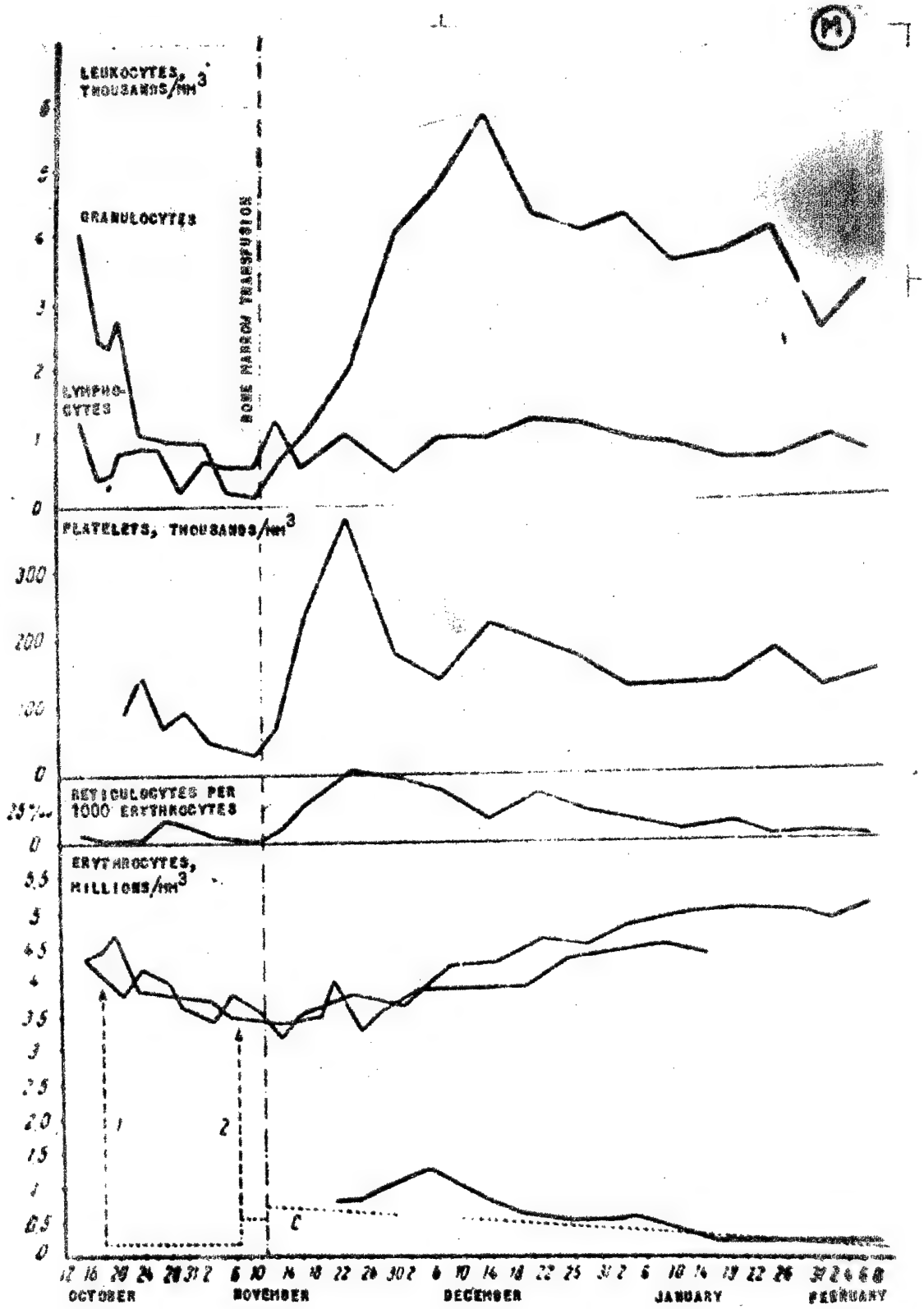


Fig. 4. Changes in the Number of Peripheral Blood Cells in Patient M.

Curve C -- Changes in erythrocytes populations produced by grafted bone marrow (determinations made according to the C antigen in the erythrocytes produced by the donor bone marrow). Arrows 1 and 2 show the times of blood transfusion.

Table 3

SERUM USED	MEDIUM FOR ERYTHROCYTES	CONCENTRATION OF ERYTHROCYTES (IN 1 MM^3)	TEMPERATURE	TIME, MIN.
ANTI-C	PHOSPHATE (PH = 7.2)	800 000	37°	60
ANTI-E	THE SAME	600 000	37°	60
ANTI-C	HUMAN AB SERUM	600 000	37°	60
ANTI-LE^A	PHOSPHATE (PH = 7.2)	600 000	37°	60

After the infusions the myelograms came back to normal both qualitatively and quantitatively. In G and M a temporary increase was observed in the hyperbasophilic cells, and in M, in addition, eosinophilia (up to 20 percent).

Subsequently, in M between 5 December and 12 December (the infusion was given 11 November), and in G, D, H, between 12 December and 19 December (the infusions were given 17 November, 20 November) a divergence of the two curves occurred: the curve of the total number of red blood cells increased, while the curve of the number of erythrocytes coming from the grafted bone marrow, decreased. It may be supposed that during this period the production of erythrocytes was resumed by the bone marrow of the patients (see Tables 4-7).

Table 4 Patient D

(Data Concerning the Blood of the Recipient and of the Donor are Presented in Table 2)

DATE	PERCENTAGE OF CELLS	
	C^+ (DONOR)	E^+ (RECIPIENT)
28/XI 1956 r.	55	NOT DETERMINED
5/XII	48	51
12/XII	44	49
19/XII	28	71
26/XII	19	78
5/I 1959 r.	19	83
17/I	6	90
31/I	PRESENT	91

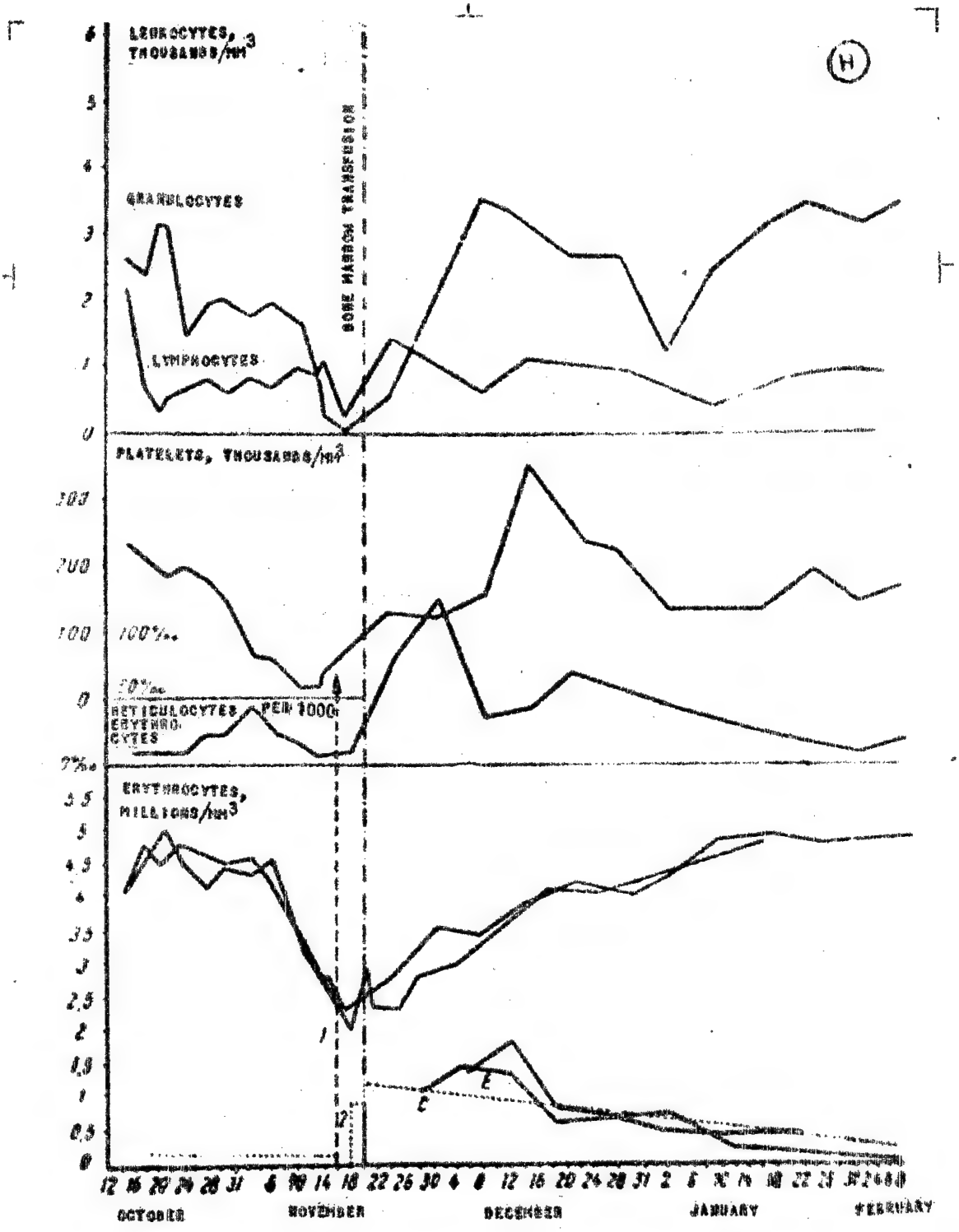


Fig. 5. Changes in the Number of Peripheral Blood Cells in Patient H.

Curves E and C show changes in the erythrocyte populations formed by bone marrow injected into the body (curve C) and the recipient's bone marrow (curve E).

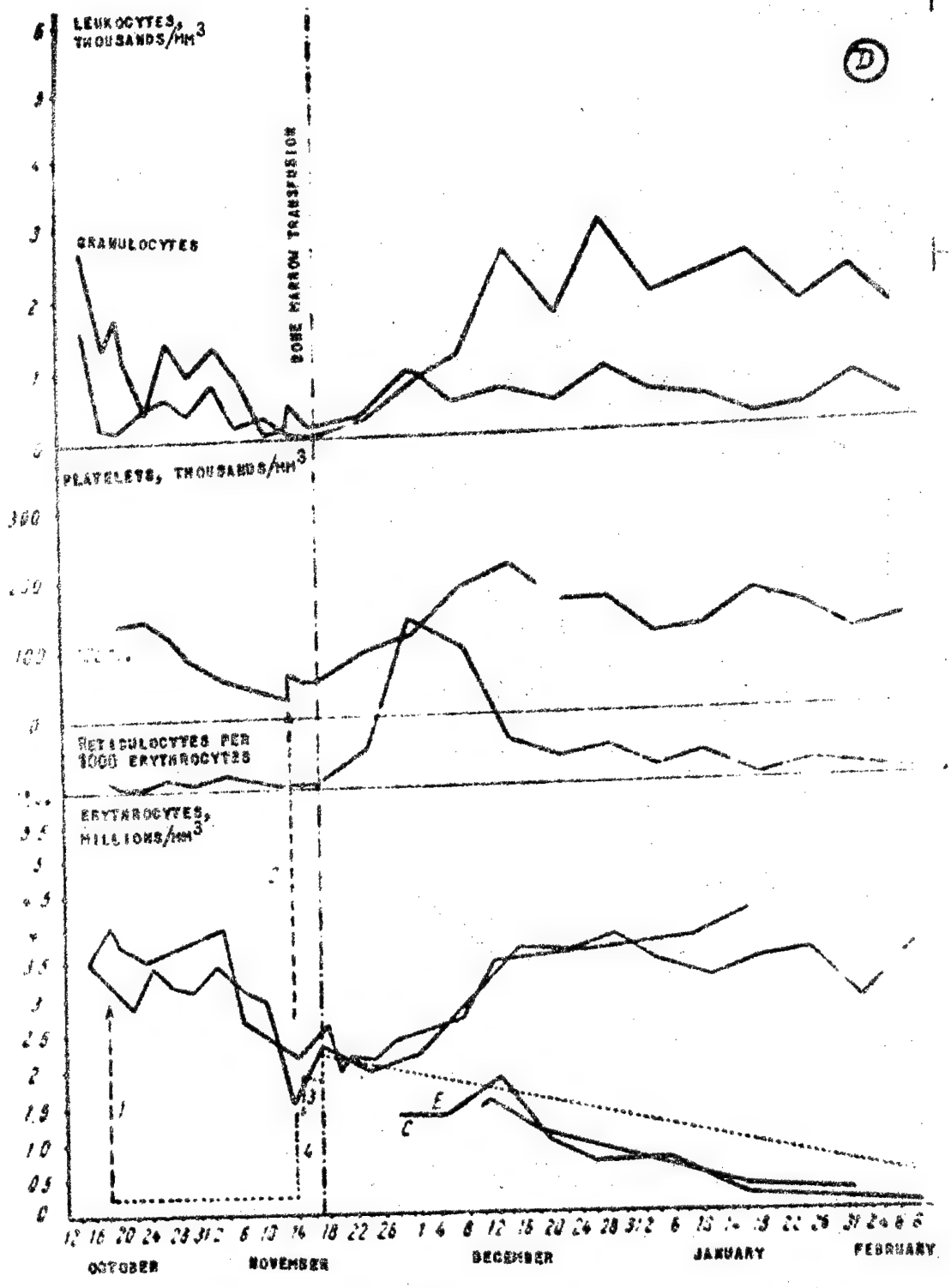


Fig. 6. Changes in the Number of Peripheral Blood Cells in Patient D.

Curves E and C showed changes in erythrocyte populations formed by bone marrow injected into the organism (curve C) and the recipient's bone marrow (curve E). The arrow 1 and the broken lines 3, 4 indicate the times blood was transfused. Arrow 2, the time of platelet transfusion.

Table 5 Patient G

(Data on the Blood of the Recipient and the Donor are Presented in Table 2)

DATE	PERCENTAGE OF CELLS	
	Le (a⁺) (DONOR)	E⁺ (RECIPIENT)
28 XI 1958 r.	12	NOT DETERMINED
5/XII	25	80
12/XII	33	69
19/XII	23	83
26/XII	14	82
5 I 1959 r.	17	89
17/I	8	92
31/I	PRESENT	92

Table 6

Patient H. Donor S.

(Data on the Blood of the Recipient and Donor are Presented in Table 2)

DATE	PERCENTAGE OF CELLS	
	C⁻ (DONOR)	E⁺ (RECIPIENT)
28/XI 1958 r	38	NOT DETERMINED
5/XII	48	55
12/XII	37	50
19/XII	17	80
26/XII	19	80
5/I 1959 r	19	85
17/I	5	89
31/I	PRESENT	91

Table 7

Patient M

(Data on the Blood of the Recipient and Donor are Presented in Table 2)

DATE	PERCENTAGE OF c^+ CELLS (DONOR)
21 XI 1958 r.	18
25/XI	25
28 XI	27
5/XII	32
12 XII	22
19 XII	13
26/XII	10
5/I 1959 r.	10
17/I	PRESENT
31/I	»

Several weeks later, variations were observed in the number of granulocytes and platelets the cause of which was hard to explain in view of the lack of methods permitting the determination of the origin of these blood cells from the donor or recipient.

The authors of the first of the articles being abstracted here discuss the problem of bone-marrow grafting in detail and the part of this method in the therapy of radiation injuries.

In dealing with the causes of an absence of an effect from grafting embryonic myeloid cells ("Meditsinskaya radiologiya," 9) into the most severely afflicted patient, V, the authors note that the onset of recovery from cytopenia could be expected no sooner than 10 days after the grafting, while the course of the disease was so severe that this period was too long for the patient. In addition, the authors believe that possibly the number of grafted embryonic cells (4,000,000,000) was inadequate. Observations of the effect of bone-marrow grafting from adults into other afflicted persons, which were not performed at the time the cytopenia was particularly pronounced, show that the mechanisms of development of compensatory hyperplasia during these periods were very effective. The following facts speak for the "assimilation" of the grafted bone marrow of adult donors: 1) the rapidity of the increase in the reticulocyte, granulocyte and platelet counts after grafting the bone marrow; 2) the discrepancy between the rapid and the considerable

rise in the curve of the granulocyte count in the blood and the lymphocyte curve, which was not very much changed after the grafting; 3) the type of platelet curve, which rapidly rose (particularly in V and M) after the grafting, and then decreased and stayed at a quite low, stable level; 4) a comparison of the curves of blood cells of patients who had been given bone marrow infusions with the curves of Patient B, who was not treated in this way. In the latter there was no reticulocyte "crisis" noted, and the number of polynucleated cells did not increase right away but rather increased slowly and reached their highest level only by the 50th day after the maximum cytopenia. The type of platelet curve was different in him also; there was no peak on it characteristic of the platelet curves in other patients.

A direct proof of the "take" of the bone marrow is the determination of the origin of the erythrocytes from the grafted bone marrow by immunological methods. It was established that the concentration of the erythrocytes in the blood is increased during the first month and that the total number of erythrocytes produced by the bone marrow is considerably greater than the number of erythroblasts injected. The authors present a number of arguments on behalf of the adequate reliability of the methods of determination of the origin of the erythrocytes. The subsequent reduction in the erythrocyte count produced by the grafted bone marrow cannot be explained by hyperhemolysis, because the Coombs test during this period was negative. Apparently, the production of these cells decreased.

The authors do not express themselves definitely concerning the possibility of a secondary syndrome after the grafting of homologous bone marrow.

In the last article, the method is described of determining the survival of erythrocytes after intravenous infusion of bone marrow. The differential agglutination according to the Würmser method was accomplished at a constant temperature by shaking a mixture of erythrocytes and serum together. Anti-C and anti-Le^a sera were used. In three patients it was possible to determine the percentage of their own cells by means of the anti-E serum. The error of this kind of determination did not exceed five percent. The data obtained with anti-Le^a, showed that this antibody can be useful for evaluating the survival of erythrocytes and that the Le^a substance remains absorbed onto the surface of cells during their entire lives.

D. E. Grodzenskiy, F. G. Krotkov

Conference of Experts on Radiochemical Methods of Analysis in the Public Health System (Geneva, 15-20 September 1958)

L. A. Kachur

The World Health Organization and the Food and Agriculture Organization, which are specialized institutions of the United Nations Organization, convoked a joint conference of the committee of experts on radiochemical methods of analysis in the public health system. Experts from England, Canada, Norway, USSR, United States of America, France, Japan, as well as consultants and representatives from Austria, United States and France were represented in the committee. The meeting of the committee took place in Geneva, in the United Nations Palace, from 15 through 20 September 1958. The meeting of the committee was carried on in English, Russian and French; the records were made in English.

The committee was confronted with the task of giving a report containing recommendations on the radiochemical and physical methods of analysis of radioactive contaminations of the environment and in man. Three reports were proposed and given on these problems: those of Doctor C. Straub, "The Investigation of the Radioactive Contamination of the Environment," of Doctor C. Comar, "Problems of Radiochemical Analysis of Agricultural Products," and of Doctor T. Stuart, "The Radiochemical Methods of Controlling Human Contamination." The material of these reports were made the basis of the decisions on these subjects in the guide book written by the committee.

For the purpose of working out the material and formulating it on various points of the report the committee was divided into a number of subcommittees of four to six persons each. Subcommittees were created to work out problems of the sources of radioactive contaminations, for an analysis of the methods of detecting radioactive contaminations in people, for methods of detecting environmental contamination, and a subcommittee on selection and recommendations of radiochemical analytical procedures. In the last subcommittee the group worked on recommendations of a specific physical apparatus for making the analyses. The reports of the subcommittees were presented for the benefit of the committee members and were discussed at the joint session of the committee of experts, after which they were considered adopted.

As the result of the work of the session, a report was

written by the experts containing a detailed description of the radiochemical methods of analysis recommended, which after approval by the Executive Committee of the World Health Organization will be printed in several languages.

Below, a brief summary of the report of the experts committee is given in the last of the variants adopted.

I. Sources of Radioactive Contamination

Obtaining information concerning the sources of radioactive contamination is essential for making out a plan of analyses of isotopes in case of radioactive contamination of the environment and of man. In the classification of the possible type of radioactive contamination consideration should be given to the following: 1) the level of activity existing in the source; 2) the quantity of activity emitted; 3) whether the emanation is the usual or chance; 4) isotopes which may be present in the source; 5) chemical and physical forms in the substance isolated; 6) an evaluation of the area affected; 7) whether there is a potential danger for the entire population or only for personnel exposed to irradiation; 8) what the nature of the environment is.

In the report the need was noted for taking into consideration the level of natural activity in the determination of the level of contamination of the environment and of man.

II. Evaluation of Contamination of the Environment

In this section general comments are given concerning an approach to the selection of the type of analysis and an evaluation of its results. An evaluation of the results is achieved by a comparison of the data obtained with the maximum permissible concentrations recommended by the International Commission on Radiological Protection for a number of isotopes in water, air and in the human body. The most widespread isotopes are mentioned, and a basis is given to the need for analyses of radioactive isotopes in the following specific substances: in air, drinking water, soil, plants and products of vegetable origin, in milk and other products of animal origin, as well as in reservoirs and products of aqueous origin. At the end of this section, a table is given with an indication of the isotopes of greatest interest in the analysis of the media listed.

III. Evaluation of Human Contamination

In the analysis of the types of analyses needed for evaluating the degree of contamination of people consideration should be given to the fact that radioactive substances

enter the body by three routes: 1) inhalation of gas particles or aerosol particles; 2) by swallowing (for example, of contaminated food); 3) by penetrating into a wound and the intact skin. The danger depends on the relative biological effectiveness of the given type of radiation. In the majority of cases (with the exception of hard gamma-emitters) internal contamination of the body is judged by the content of isotopes in the excretions, chiefly in the urine. In the event of the presence of insoluble isotopes a stool analysis is essential for the evaluation of the internal contamination. An analysis of radon in the exhaled air provides a determination of the initial level of radium in the body. The measurement of the blood activity is necessary for obtaining information on various isotopes contained in the body. For these purposes analyses of the gastric juice, tissues, nasal excretions and saliva may be used. Radioactivity detected indicates the need for taking prompt measures, but does not give any accurate information concerning the quantity of isotopes contained in the body. In order to determine the level of internal contamination it is essential to know the relationship between the rate of excretion of the given isotope and its concentration in the body. The difficulties of measuring the internal contamination according to the excretion products are associated also with the determination of the low concentrations of isotopes in the excretions and with the need for taking into consideration the natural background of the excretions. Extrinsic direct methods of measuring the activity are utilized for hard beta- and gamma-radiation (Ra^{226}, Cs^{137}, I^{131} and others).

At the end of the section, a summarized table is given with an indication of the most widespread isotopes and their maximum permissible concentrations in biological media (urine, stool, blood, thyroid gland, etc.).

IV. Methods

In this section material is contained on three problems: 1) the technique of selection and preparation of the samples; 2) the analytic radiochemical procedures for determining the isotopes; 3) the physical apparatus utilized for making the analyses.

The technique of selection of the samples. The analysis is reliable when the sample is correctly and carefully selected. The selection of the samples can be done either once or regularly. The former characterizes static conditions; the latter provides information of dynamic nature and makes it possible to evaluate the contamination

of the environment or of man for a certain interval of time. The sample should be characteristic of the composition of the medium being analyzed. The size of the sample required is determined by the activity level and the quantity needed for the radiochemical assay. In consideration of these principles the characteristics of taking specimens from a number of samples are analyzed. For air and other gases the characteristics are noted of selecting the samples in filtration, electrostatic precipitation, separation and sedimentation methods. The characteristic features and difficulties are analyzed in taking samples of drinking, fresh and sea water, sewage and industrial water wastes as well as samples of bottom mud.

Radiochemical methods of analysis of isotopes. The criteria for choosing the analytical method are the following: 1) high degree of sensitivity; 2) accuracy; 3) rapidity; 4) economy with respect to material and apparatus; 5) possibility of utilization for analyses in various media. For the purpose of specific analysis two methods were recommended for the purpose of specific analysis with respect to the majority of isotopes; one, the most accurate and laborious; the other, more rapid but less accurate. The results of the analysis are reported in customarily accepted units (according to the instructions of the International Commission on Radiological Protection).

The specific methods of analyses proposed in the report include the majority of isotopes characteristic of radioactive contamination of the environment which are really dangerous for human health. In the report analyses are given for the following isotopes: H^3, Sr^{89} and Sr^{90}, Ru^{103}, I^{131}, Cs^{137}, Po^{210}, Rn, Ra, Th^{230} and Th^{232}, U, Pu^{239}. Hereby, a determination is given of each of the isotopes in various biological media -- in the urine, blood and others, as well as in water, soil, plants, in the milk and other products of vegetable and animal origin. In addition, analyses are given for considerable unidentified alpha- and beta-activity.

Physical methods of measuring radioactivity. The following material is contained in the report: 1) on the standard radiometric equipment; 2) gamma-spectrometry, and 3) on in vivo measurements in the case of internal contamination of man.

In the section on standard apparatus the main requirements are presented which are made on radiometric apparatus in radiochemical methods of analysis. In the light of these requirements ionization chambers, Geiger end-type counters, proportional counters, which are usually connected up

according to an anticoincidence system, as well as alpha- and gamma-scintillation counters. The need and conditions of standardization of the apparatus are also noted. Gamma-spectrophotometry is considered the greatest achievement in radiometric apparatus in recent years, and is particularly essential for analytical chemists, because a rapid direct measurement of activity with the gamma-spectrometer without preliminary treatment of the sample, and determination of substances in different states of aggregation with a high degree of sensitivity, stability and accuracy are possible.

The apparatus for the direct external measurement of the activity of an element or compound is designed basically for a gamma-radiation determination. In the modern state of technics it can be used for the detection of certain beta-emitters by means of their braking radiation. The following may be recommended as measuring apparatus: a) ionization gas-filled chambers of large volume; b) liquid and solid scintillators. In in vivo measurements three questions may be of interest: 1) the measurement of the activity of the entire body; 2) the determination of the activity localized in a certain part of the body; 3) the spectral composition and the distribution of the energy emitted by the object. The selection of the apparatus depends on the nature of the problem being solved.

From what has been presented it is evident that the subject of the report is of current importance. The need has matured for a guide-book on problems of radiochemical methods of analysis. Specialists working in the field of medical radiology will find useful and essential information in the report both for the organization of this work and for its practical development. In the report material has been put together well in the matter of giving a sound basis to selective samples characteristic of radioactive contamination of a given medium and the practical accomplishment of analyses of them. A large bibliography is given in the report.

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